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OPEN PEER REVIEW

# Ginger Extract Modulates the Production of Chemokines CCL17, CCL20, CCL22, and CXCL10 and the Gene Expression of Their Receptors in Peripheral Blood Mononuclear Cells from Peptic Ulcer Patients Infected with *Helicobacter pylori*

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## About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

## Contents

1 **Original submission** Referee 1, Referee 2, Referee 3, Associate Editor, Editor-in-Chief ..... 2
2 **Revision 1** Referee 4, Associate Editor, Editor-in-Chief ..... 4
Authors' reply to reviewers ..... 5
Reviewer and editor comments ..... 11
3 **Revision 2** Referee 4, Associate Editor, Editor-in-Chief ..... 12
Authors' reply to reviewers ..... 13
Reviewer and editor comments ..... 14
4 **Revision 3** Referee 4, Associate Editor, Editor-in-Chief ..... 15
Authors' reply to reviewers ..... 16
Reviewer and editor comments ..... 17

## 1 Original submission

5 comments · 27 Feb 2020 – 2 Aug 2020

### Referee 1 Reviewer

MAJOR REVISION 27 Feb 2020

Dear author;

Thank you for choosing JJNPP for publishing your manuscript. This article describe very interesting results onto chemokines modulations by Ginger extract, in a context of infection by H. pylori.
This article is very well-written but I recommend to perform an global English verification.
Some issues remain:

- Bacteria names have to be indicated in italic
- Authors have to give more information about the choice of their Hp-infected cohort. Why did they not include cancer-associated PBMCs?
- In abstract, it is preferred to include multiplication/division factor in place of p-values.
- In introduction and discussion, authors have to discuss about the impact of their extract on bacterial microbiota, as these microbial communities have been described as associated to H. pylori correa cascade. (See Pichon et al. Healthcare)
- Can the authors justify the differential effect of Gignger extracts on CagA+ andCagA and strains ?
- About RNA extraction, can the authors specify the percentage of the Agarose for electrophoresis? For the RT-PCR can the authors indicate how the addition of RT enzyme was performed (automatic or manual)?
- For statistical analysis, if the authors specify that p-values considered as significant in 0.007, do not present results of >0.007.
- For Figure, present only significant results and not p>0.007 values. Moreover it could be interesting to present comparison of ginger-treated results and control results.

Good Luck!

### Referee 2 Reviewer

MINOR REVISION 19 Jul 2020

Dear author;

Thank you for choosing JJNPP for publishing your manuscript. There are some points which should be corrected;

1- What the meaning of ".....and their receptors by peripheral blood mononuclear cells from Helicobacter pylori"?

2- Newer references are needed for methods applied in your project.

Good Luck!

### Referee 3 Reviewer

MAJOR REVISION 2 Aug 2020

Dear author;

Thank you for choosing JJNPP for publishing your manuscript. There are some points which should be corrected;

1. The results are very poorly presented. It is recommended to use a statistical expert to improve the reporting of results.
2. The use of some statistical tests such as paired t-test and chi-square is not seen in the charts. It is wrong to use P<0.007 or P<0.02 statistical terms.
3. It is recommended that for each chemokine and receptor expression, the results of the three groups (NHC, HAS, PU) be shown on a graph and only the necessary comparisons be made.
4. Use the * and # symbols for comparisons with the control group and PU crude, and only at a significance level of P<0.05.
5. The extraction method is incomplete. Is ethanolic extract taken or hydroalcoholic? If the hydroalcoholic extract is taken, the ratio of water to ethanol should be written and the amount of powder and solvent should be mentioned. The time for extraction maceration and the percentage of extraction efficiency should be mentioned. Write a reference for the extraction method.
6. What is the reason for selecting 20 patients for each group? Reference should be made to the sample size or method of obtaining the sample size.
7. Correct spelling of "Abitale Hospital" as"Abitaleb Hospital"
8. Correct "concentrations of the extract. 20-, 10- and ..." as"20, 10"
9. The unit of extract concentrations should be modified throughout the text as "µg/ml"

Good Luck!

**Associate Editor** MAJOR REVISION 2 Aug 2020

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author.
According to the comments, the document needs major revision.
Kind regards,

**Editor-in-Chief** MAJOR REVISION 2 Aug 2020

Dear Author,
The reviewers' comments are mentioned. Please correct the details as mentioned above carefully according to reviewers' request.
Thanks.

2

# Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

## Dear Professor Hemmati

## The Editor of Jundishapur Journal of Natural Pharmaceutical Products

Salam and Good time.

Thank you very much for your email (dated 2ed August 2020) enclosing the reviewers comments on our manuscript entitled: **"Ginger extract modulates the expression of chemokines CXCL10, CCL17, CCL20, CCL22 and their receptors by peripheral blood mononuclear cells from Helicobacter pylori-infected patients with peptic ulcer [ID: 100824].** We have considered the reviewers comments and the corrections and modifications were done according to their comments as highlighted in the main text of manuscript and explained follow:

**Reviewer #1:**

**Point #1:** Bacterial name have to be indicated in *italic.*

**Response:** The bacterial name and its abbreviation (*HP*) indicated in *italic* format in throughout of manuscript.

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**Point #2:** Authors have to give more information about the choice of their HP-infected cohort. Why did they not include cancer-associated PBMCs?

**Response:** The information about the choice of their HP-infected PU patients was provided in the material and methods section (Please see Pages 5 and 6: L152-L162). The information about the choice of their HP-infected asymptomatic subjects (HAS) was provided in the material and methods section (Please see Page 6: L162-L168). The main purpose of this project was to investigate ginger extract effect on expression of selected chemokines and chemokines receptors by PBMCs from *HP*-infected PU patients to clear the alteration of selected chemokines and

chemokines receptors in HP-infected PU patients and to evaluate the ginger capability in the modulation of these changes. We hope to study the cancer patients in future projects.

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**Point #3:** In abstract, prefer to include multiplication/division factor in place of p-values.

**Response:** In abstract, the multiplication/division factors were added in place of p-values. (Please see Page 2: L43-L53).

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**Point #4:** In introduction and discussion, authors have to discuss about the impact of their extract on bacterial microbiota, as these microbial communities have been described as associated to H. pylori correa cascade. (See Pichon et al. Healthcare)

**Response:** In introduction and discussion the impact of the ginger extract on the stomach bacterial microbiota was described according to study of Pichon et al. in Healthcare (Please see Page 4: L110-L123 and Page 15: L442-L453).

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**Point #5:** Could the authors justify the differential effect of Ginger extracts on CagA+ and CagA- strains ?

**Response:** The explanations concerning the justification of the differential effect of Ginger extracts on CagA+ and CagA- strains were added to discussion (Please see Page 15: L454-L461).

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**Point #6:** About RNA extraction, could the authors specify the % of the Agarose for electrophoresis? For the RT-PCR could the authors indicate how the addition of RT enzyme was perfomed (automatic or manual)?

**Response:** We used 1.5% of the Agarose for electrophoresis (Please see Page 7: L199). For the cDNA Synthesis we used the ready Pars-Tous cDNA Synthesis Kit to form cDNA and the protocol was performed according manufacture guidelines (Please see Page 7: L201-L203).

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**Point #7:** For statistical analysis, if the authors specify that p-values considered as significant is 0.007, do not present results >0.007.

**Response:** According to reviewer comment, only the p-values that were significant were mentioned.

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**Point #8:** For Figure, present only significant results and not p>0.007 values. Moreover it could be interesting to present comparison of ginger-treated results and control results.

**Response:** Only significant P values were exhibited in Figures. Moreover, the comparisons of ginger-treated results and control results were exhibited in Figures and mentioned in the Results section.

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## Reviewer #2:

**Point #1:** In title phrase..."and their receptors by peripheral blood mononuclear cells from Helicobacter pylori".. What means?

**Response:** The Title was revised to be clearer (Please see Page 1: L2-L3).

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**Point #2:** Newer references for methods applied in project is essential.

**Response:** Newer references were provided for methods (Please see Page 5: L134-L135 reference 34 and Page 5: L138-L140 reference 37 and 38 and Page 6: L177-L179 reference 43).

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**Reviewer #3:**

**Point #1:** The results are very poorly presented. It is recommended to use a statistical expert to improve the reporting of results.

**Response:** All results were rechecked and improved under the supervision of a statistical expert colleague as a co-author (Dr Sheikh Fathollahi Assistant Professor of Biostatistics).

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**Point #2:** The use of some statistical tests such as paired t-test and chi-square is not seen in the charts. It is wrong to use $P<0.007$ or $P<0.02$ statistical terms.

**Response:** All P values were mentioned according to reviewer comment.

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**Point #3.** It is recommended that for each chemokines and receptor expression, the results of the three groups (NHC, HAS, PU) be shown on a graph and only the necessary comparisons be made.

**Response:** According to ten essential binary comparisons in chemokine and ten binary comparisons in chemokine receptors in each group, presenting the results of three groups in a graph, as means presenting 60 comparisons in 1 graph, and this leads to a lack of understanding of the results.

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**Point #4.** Use the * and # symbols for comparisons with the control group and PU crude, and only at a significance level of $P<0.05$.

**Response:** P value, are corrected in text and figures and in order to control the type I error in hypothesis testing, Bonferroni correction was also applied that revealed the P values ≤0.005 were considered significant and we presented only significance level in graph and text.

---

**Point #5:** The extraction method is incomplete. Is ethanolic extract taken or hydroalcoholic? If the hydroalcoholic extract is taken, the ratio of water and ethanol should be written and the amount of powder and solvent should be mentioned. Time for maceration for extraction. Write the percentage of extraction efficiency. Write a reference for the extraction method.

**Response:** The extraction method was complete and reference for the extraction method was added in method. (Please see Page 5: L134-L140), references 34-38.

---

**Point #6:** What is the reason for selecting 20 patients for each group? Reference should be made to the sample size or method of obtaining the sample size.

**Response:** We selected 20 participants for each group, according sample size formula, sample size was added to methods. (Please see Page 5: L150).

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**Point #7.** Correct spelling of Abitale Hospital: Abitaleb Hospital

**Response:** Spelling of Abitaleb Hospital, was Corrected. (Please see Page 5: L153),

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**Point #8.** Correct writing concentrations of the extract. 20-, 10- and ... to 20, 10

**Response:** Were corrected from, 20-, 10- and ... to 20, 10 in text and figures.

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**Point #9.** The unit of extract concentrations should be modified throughout the text: µg/ml

**Response:** The unit of ginger extract concentrations was modified throughout the text and graph from µg to µg/ml in text and figures.

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Yours Sincerely
Abdollah Jafarzadeh (Professor, Ph.D)
Department of Immunology
Medical School
Kerman University of Medical sciences Kerman
I.R. IRAN

## 2 Revision 1

3 comments · 4 Oct 2020 – 10 Oct 2020

### Referee 4 Reviewer

MAJOR REVISION 4 Oct 2020

Dear author;

Thank you for all the corrections. But there are still some points which should be corrected;

1-Due to the large number of variables, the results should be presented as a table.Three groups should be presented in the rows of the table and the mean and standard deviations of the studied variables in the columns of the table and a significant level below each column.
2- Provide the name of the statistical tests used and just writing the general linear model is not enough.
3- In the results section, writing three significant levels is not necessary, if p1=0.001 and p2=0.002 and p3=0.003 be written like this P<=0.003 .
4-Line 231 should be written as:The ratio of men/ women was the same in the three groups(12/8).
5-On the line 150 in the sample size formula: mean and standard deviation of the variable used, alpha and beta should also be reported. The sample size formula should be used to compare the means of the three groups, not the two groups.

Good Luck!

### Associate Editor

MAJOR REVISION 10 Oct 2020

Dear EIC,
The manuscript is studied, and there are some comments which should be addressed by the author.
According to the comments, the document needs major revision.
Kind regards,

### Editor-in-Chief

MINOR REVISION 10 Oct 2020

Dear Author,
Your article is reviewed by the reviewers carefully. According to their comments, this document needs minor revision. Please follow their comments as requested.
Thanks.

3

# Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**Dear Professor Hemmati**
**The Editor of Jundishapur Journal of Natural Pharmaceutical Products**
Salam and Good time.
Thank you very much for your email (dated 10th October 2020) enclosing the reviewer comments on our manuscript entitled: “Ginger extract modulates the expression of chemokines CXCL10, CCL17, CCL20, CCL22 and their receptors by peripheral blood mononuclear cells from Helicobacter pylori-infected patients with peptic ulcer [ID: 100824]. We have considered the reviewer comments and the corrections and modifications were done according to their comments as highlighted in the main text of manuscript and explained follow:
1-Due to the large number of variables, the results should be presented as a table. Three groups should be presented in the rows of the table and the mean and standard deviation of the studied variables in the columns of the table and a significant level below each column.
Answer
The results were presented in one Table, according to reviewer comment. Because the comparisons are made through the PBMC cultures, the P-values are written at the end of each row. Pages 24/25.
2- Provide the name of the statistical tests used and just writing the general linear model is not enough.
Answer
Name of the statistical tests was added and highlighted. Page: 8, Lines 224/225
3- In the results section, writing three significant levels is not necessary, if p1=0.001 and p2=0.002 and p3=0.003 be written like this P<=0.003 .
Answer
In the results section the P values were merged according to reviewer comment: Pages 8-11, Lines 240, 242, 246, 250, 257, 263, 270, 271, 275, 277, 281, 285, 295, 297, 312, 327 and 330.
4-Line 231 should be written as: The ratio of men/ women was the same in the three groups(12/8).
Answer
This line was replaced according reviewer comment and highlighted. Page: 8, Lines 232/233
5-On line 150 in the sample size formula: mean and standard deviation of the variable used, alpha and beta should also be reported. The sample size formula should be used to compare the means of the three groups, not the two groups.
It is better not to write.
Answer
The *alpha* and *beta* were added according reviewer comment. Available sample size formulas are just to compare a maximum of two groups and no more. Therefore, when the sample size is calculated for comparing two groups, the result can be simply generalized to more than two groups. Page: 5, Lines 150/151
Dr Abdollah Jafarzadeh
Department of Immunology
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Kerman University of Medical sciences Kerman
I.R. IRAN

*[Translated from Persian by the Brieflands editorial office.]*

## 3 Revision 2

3 comments · 12 Oct 2020 – 13 Oct 2020

### Referee 4 Reviewer

MINOR REVISION 12 Oct 2020

Dear author;
The requested corrections have been made but there are only four points left:

1-In Table 2, only intra group comparisons were made and there is no between group comparisons .

2- ANOVA should be written on line 225

3- In the abstract , merge the p- values similar to the results.

4-In response to the sample size formula, the author answers that the sample size is calculated with the maximum difference between the two groups, while the sample size should be calculated with the minimum difference.

Good Luck!

### Associate Editor

MINOR REVISION 13 Oct 2020

Dear EIC
The reviewer(s) have recommended publication, but also suggest some minor revisions to the manuscript. Therefore, I invite the author to respond to the reviewer(s)' comments and revise his manuscript.
Kind regards,

### Editor-in-Chief

MINOR REVISION 13 Oct 2020

Dear Author,
Your article is reviewed by the reviewers carefully. According to their comments, this document needs minor revision. Please follow their comments as requested.
Thanks.

4

# Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

**Dear Professor Hemmati**

**The Editor of Jundishapur Journal of Natural Pharmaceutical Products**

Salam and Good time.

Thank you very much for your email (dated 13th October 2020) enclosing the reviewer comments on our manuscript entitled: “Ginger extract modulates the expression of chemokines CXCL10, CCL17, CCL20, CCL22 and their receptors by peripheral blood mononuclear cells from Helicobacter pylori-infected patients with peptic ulcer [ID: 100824]. We have considered the reviewer comments and the corrections and modifications were done according to their comments as highlighted in the main text of manuscript and explained follow:

1-In Table 2, only intragroup comparisons were made and no between group comparisons
Answer: In Table 2, between groups comparisons were made and highlighted, page 24 and 25.
2- ANOVA should be written on line 225
Answer : ANOVA was written on line 226 page 8 and highlighted.
3- In the abstract, merge the p- values similar to the results
Answer: In the abstract, merged the p values like to the results and highlighted, *Page 2 line 47,50,53*.
*4-In response to the sample size formula, the author answers that the sample size is calculated with the maximum difference between the two groups, while the sample size should be calculated for the minimum difference.*
*Answer: sure, we calculated the sample size according the minimum difference.*

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## 4 Revision 3

3 comments · 14 Oct 2020 – 14 Oct 2020

### Referee 4 Reviewer

ACCEPT 14 Oct 2020

Dear author;

It is now accepted, but please compare the groups in Table 2 as well.

Best regards!

### Associate Editor

ACCEPT 14 Oct 2020

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

### Editor-in-Chief

ACCEPT 14 Oct 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks