Iranian Journal of Pharmaceutical Research
Volume 22 · Issue 1 · e136173

OPEN PEER REVIEW

In vitro Evaluation of the Potent Antileishmanial Activity of *Ferula tabasensis* Alone or in Combination with Shark Cartilage Extract Against the Standard Iranian Strain of *Leishmania major* (MRHO/IR/75/ER)

Shaylin Saraei, Narges Soozangar, Mansour Miran, Fatemeh Ghaffarifar, Behnam Mohammadi-Ghalehbin, Soheila Molaei, Shabnam Asfaram

SUBMITTED	REVISED	ACCEPTED	DOI
12 Mar 2023	28 May 2023	6 Jun 2023	10.5812/ijpr-136173

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** Associate Editor 2
2 **Revision 1** Referee 1, Referee 2, Associate Editor 3
Authors' reply to reviewers 4
Reviewer and editor comments 8
3 **Revision 2** Associate Editor 9
Authors' reply to reviewers 10
Reviewer and editor comments 11

1 Original submission

1 comment · 9 Apr 2023

Associate Editor

MAJOR REVISION 9 Apr 2023

Dear authors
The referees raised some questions or proposed some justifications. It would be better to make the corresponding corrections, if possible; otherwise, a response could be sent.

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.

Dears Author Support Center,
Brieflands

Thank you very much for your email regarding our article entitled "***In vitro* evaluation of the potent antileishmanial activity of** *Ferula Tabasensis alone or in combination with shark cartilage extract* **against standard Iranian strain of *Leishmania major* (MRHO/IR/75/ER) ".**
I would like to thank you for your comments and suggestions. We have made almost all the changes suggested by you and the respected reviewers. I hope the revised article is now accepted for publication Innovative Journal of Pharmaceutical Research. Changes are marked with yellow highlights in the revised text. In addition, we edited the English language of the text. These changes are marked with a red pen.

Reviewer 1:
The Manuscript entitled "In vitro evaluation of the potent antileishmanial activity of Ferula Tabasensis alone or in combination with shark cartilage extract against standard Iranian strain of L. major (MRHO/IR/75/ER) "is an innovative investigation that evaluates the effect of Ferula Tabasensis alone or in combination with shark cartilage extract on L. major in in vitro. Please take to consideration some comments below to improve the quality of MS since some points have to clarify:

The authors would like to thank the Reviewer for his/her helpful scientific comments.

Q1: *in title: Where you write the name of genus and species at the first time, please write them in the full name. (Leishmania major)*

Answer: It has been added and highlighted.

Q2. *In abstract: In whole text of the submitted manuscript it is necessary to check and write correct units of measure according to International System of Units. (For instance, Row 21). The results part of the abstract needs to be edited. Please add shark cartilage in the keywords.*

Answer: The manuscript has been updated based on scientific reviewer request and highlighted. The units of measure are µg/mL, all of abstract re-edited, shark cartilage added in the keywords.

Q3. *Introduction: In the whole manuscript, the type of extract should be specified (Row 64 and …).*
Answer: OK, the type of extracts and fractions have been edited and highlighted.

Q4. *Materials and Methods: Please write J774A.1 cell line correctly in the manuscript (Row 90, 162, Table4, …). Please state if you have used a MTT solvent (Row 112) also explain about the negative control (Row 157). In the MTT Assay for Promastigote Viability method section, please state the volume of compound encountered and describe whether you performed centrifugation (how many times and when) or not.*

Answer: The manuscript has been updated based on scientific reviewer comments and highlighted. J774A.1 re-written in whole text. PBS as MTT solution has been used as

control. Also, MTT assay described completely in its section.

Q5. *Results: Table 2 shows the effect of ShCE (200 µg/mL) in combination with F. Tabasensis at IC50 concentration on Leishmania parasite; Here, the question arises as to how the concentration of Amphotericin B and Glucantime were chosen and whether they are in combination with ShCE (200 µg/mL) or not. In line 111, you state that the evaluation was done after 72 hours, but in the results it also shows exposure times of 24 and 48 hours, please explain. Is the term "treatment" correct here? (lines 256, 288, 337) Please explain.*

Answer: As known, the effect of amphotericin B on promastigotes is very high. In contrast, the effect of glucantime is low. In our previous studies and other researchers, amphotericin B is usually selected with low dosage (e.g. 1 µg/mL) and glucantime with high dosage, but in the present study, considering that the IC50 of the extracts and fractions were compared with the IC50 of these reference drugs, the concentrations of these reference drugs are also was selected like extracts and fractions.
The dose of 200 shark was selected according to our previous study (ref. 19).
After determining the IC50 of drugs, extracts, and fractions, the combination implied an almost equal volume (for example for 100 µg/mL volume: 50 µg/mL shark extract at 200 µg/mL + 50 µg/mL of extract or fraction at IC50 concentration).
The evaluation after 24, 48, and 72 h was brought in the separation part entitled Microscopic inhibition test. The MTT assay was conducted after 72 h.
The term "treatment" was replaced with “exposure”.

Q6. *Discussion: Please write the reference of line 337.*

Answer: Ok, it is added.

Reviewer 2:
Dear Editor
The article titled "In vitro evaluation of the potent antileishmanial activity of Ferula Tabasensis alone or in combination with shark cartilage extract against Iranian standards strain of L. major (MRHO/IR/75/ER)" was examined. The desired points are mentioned line by line and highlighted in the text of the article. In general, the following can be mentioned in the article:

The authors would like to thank the Reviewer for his/her helpful scientific comments.

Q1. *In the working method section, the effect of compounds after 72 hours has been checked. If 24 and 48 hours later are also mentioned in the results section.*

Answer: Thank you for your constructive comment. The missed section "Microscopic inhibition test" has been added.

Q2. *Tables one and two, in which the average number of promastigotes is mentioned, and it is said that it is related to the results of MTT, cannot be the result of this test because only the percentage of cell survival can be calculated in this way. In addition, the calculation formula is incomplete and the amount of optical absorption of the blank is not mentioned.*

Answer: OK, thanks for your attention. The text of Tables 1 and 2 were mentioned separately both in methods and results. The calculation formula of viability has been corrected.

Q3. *In the results section of the amastigote form, it is mentioned that 42.5% of the macrophages in the negative control group were infected with parasites. This is a low percentage. (In most studies, usually 75% or more of the macrophages are infected). This low percentage can be caused by the poor quality of the cell line or the insufficient number of stationary promastigotes.*

Answer: Practically, this strain of *L. major (MRHO/IR/75/ER)* does not have high infectivity in macrophage cells and animal models recently, so that ulcers in balb/c mice formed after the fourth passage. However, the infectivity rate in macrophages in our study was higher than in other recent studies:

1. **Tavakoli P, Ghaffarifar F, Delavari H, Shahpari N. Efficacy of manganese oxide (Mn2O3) nanoparticles against Leishmania major in vitro and in vivo. Journal of Trace Elements in Medicine and Biology. 2019 Dec 1;56:162-8.**
2. **Hesami D, Ghaffarifar F, Dalimi A, Dayer MS, Nasiri V, Sheikh S. Investigating in Vivo and in Vitro Effects of Ethanolic and Aqueous Extracts of Myrtle (Myrtus communis) on Leishmania major. Iranian Journal of Parasitology. 2021 Oct;16(4):641**.

Q4. *Another important point is to calculate the number of amastigotes inside the macrophages compared to the negative control group, based on which the IC50 is calculated, not the percentage of infected macrophages. Therefore, the results of Table 3 are also ambiguous.*

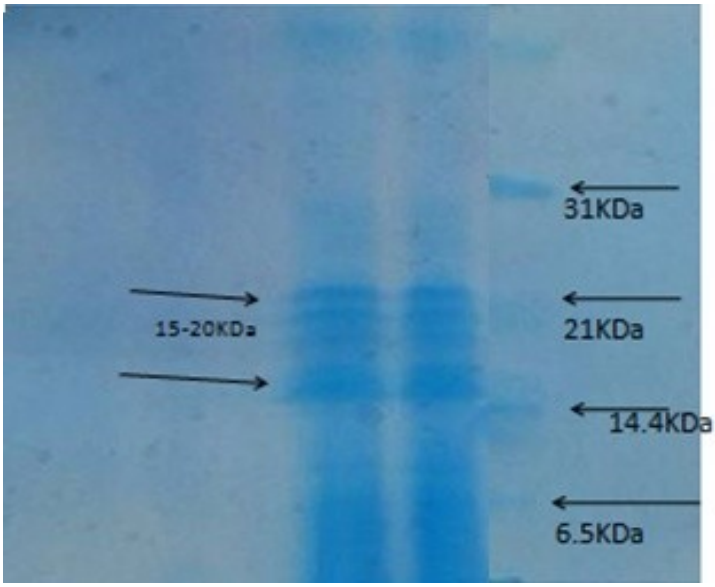
Answer: The IC50 of amastigotes was achieved on the number of amastigotes inside the macrophages, so these results were added to Table 3.

Other refrees comments which a PDF file has been answered them one by one in manuscript. Also we reply for all comments in pdf file. This PDF file has been attached as a

Research data.

-Results of SDS-PAGE and isolated proteins with molecular weight are not shown:





2 Revision 1

3 comments · 14 May 2023 – 23 May 2023

Referee 1 Reviewer

ACCEPT 14 May 2023

In the "Microscopic inhibition test" section, please mention the parasite counting method.

Referee 2 Reviewer

MINOR REVISION 20 May 2023

The manuscript entitled "Clinical Features, Laboratory Findings,and the Impact of Concomitant ParasiticInfection in Patients Hospitalized withCOVID-19 in the northern Persian Gulf,Bandar Abbas City, Southern Iran in2020-2021" provides valuable data regarding parasitic infection in COVID-19 patients.
Note:
*The genus and species of Leishmania major should be written in full in the keyword.

*Please explain why, in the Promastigote assay section,
promastigotes in the logarithmic phase were used, but in the flow cytometry assay, Leishmania promastigotes at the stationary stage were used.

*Explain the meaning of this sentence: "Apoptosis occurrence in amastigote cells inside infected macrophages was more than in promastigotes and non-infected macrophages."

Associate Editor

MINOR REVISION 23 May 2023

Dear authors
The manuscript was reviewed by the referees, and they would like you to answer two minor questions.
Please check the comments and give appropriate responses.

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.

R2:

Dears Author Support Center,
Brieflands

Thank you very much for your email regarding our article entitled "***In vitro* evaluation of the potent antileishmanial activity of** *Ferula Tabasensis alone or in combination with shark cartilage extract* **against standard Iranian strain of *Leishmania major* (MRHO/IR/75/ER) ".**
I would like to thank you for your comments and suggestions and also the reviewers for their valuable comments for the second edition. We have made almost all the changes suggested by you and the respected reviewers. I hope the revised article is now accepted for publication Innovative Journal of Pharmaceutical Research. Changes are marked with yellow highlights in the revised text. In addition, we edited the English language of the text. These changes are marked with a red pen.

Reviewer 1:

Q1: *In part of "Microscopic inhibition test" please mention the parasite counting method.*

Answer: Thanks for this comment. It has been added and highlighted both in methods and results.

Reviewer 2:

Q1. *The genus and species of Leishmania major should be written in full in the keyword.*
Answer: Thank you for your constructive comment. It is modified and highlighted.

Q2. *Please explain why in the Promastigote assay section*
Promastigotes at logarithmic phase were used, but in the flow cytometry assay, Leishmania was used in promastigote at the stationary stage.

Answer: Thanks for your careful attention. In the promastigote assay and flow cytometry section, we used the logarithmic stage of promastigotes. It is modified and highlighted.

Q3. *Explain the meaning of this sentence? Apoptosis occurrence in amastigote cells inside infected macrophages was more than in promastigotes and non-infected macrophages.*

Answer: The percentage of apoptosis (early+late) was more in infected macrophage cells compared to promastigotes and non-infected macrophages. **It is re-written and highlighted.**

3 Revision 2

1 comment · 4 Jun 2023

Associate Editor

ACCEPT 4 Jun 2023

Dear authors
Thank you for making modifications according to the referee's comments. I would like to suggest that the article be accepted for publication in the journal.