Iranian Journal of Pharmaceutical Research
Volume 23 · Issue 1 · e147234

OPEN PEER REVIEW

Synergistic Interaction of Chloroquine with *Artemisia kopetdaghensis* Semipolar Extract Against *Plasmodium berghei*: Histopathological and Immunological Studies in a Mouse Model

Roya Amirian, Mustafa Ghanadian, Hamed Fouladseresht, Azar Baradaran, Seyed Mohammad Abtahi, Bahareh Basirpour, Maryam Fattahian, Seyed Mahmoud Mousavi, Parastoo Hassani-Abharian, Hajar Shabandoust, Seyedamirmehdi Hejazi Dehaghani, Seyed Hossein Hejazi

SUBMITTED	REVISED	ACCEPTED	DOI
25 Mar 2024	7 Jul 2024	3 Aug 2024	10.5812/ijpr-147234

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 2
2 **Revision 1** Referee 1 3
Authors' reply to reviewers 4
Reviewer and editor comments 7

1 Original submission

1 comment · 8 May 2024

Referee 1 Reviewer

MAJOR REVISION 8 May 2024

- Please review the English throughout the manuscript (some parts need revision).
- In the keywords, it would be better to add Plasmodium berghei.
- Why was a semipolar extract (chloroform: acetone solvent system) used for this study? Please clarify.
- As sesquiterpene lactones are usually responsible for the antimalarial effect, why was the extract standardized based on flavonoids?
- In the results section, it is mentioned that " The plant extract was simultaneously analyzed in phytochemistry lab to identify the STLs previously reported", with a reference to a previous study. It is recommended to mention this in the methods section if it was also done for this study.
- In the results section, it is mentioned that " The plant extract was simultaneously analyzed in phytochemistry lab to identify the STLs previously reported", with a reference to a previous study. It is recommended to mention this in the methods section if it was also done for this study.
- In the discussion, there is no mention of the presence of possible compounds in the selected extract, considering the effectiveness of the results.

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 Editor-in-chief

The authors sincerely thank the reviewer for constructive criticisms and valuable comments, which were of great help in revising the manuscript. The manuscript was checked. All the comments were addressed according to the referee's team point by point and highlighted in the text.

Reviewers' Comments:

Reviewer 1:
1- Kindly please check whole manuscript for English revision (in some parts needs revision).

Response: it was done.
2- In keywords it is better to add Plasmodium berghei.

Response: It was added.
3- Why semipolar extract (chloroform: acetone solvent system) used for this study? please clarify.

Response: It was clarified as follows and added to the introduction.

Artemisia kopetdaghensis is a conspicuous aromatic plant characterized by the occurrence of a broad range of sesquiterpene lactones (STLs) as well as methoxylated flavones which are previously identified form its semipolar extract(1, 2). Moreover, it has been well established that STLs and flavones exhibit remarkable biological effects including antimalarial(3-5), cytotoxic(6), antibacterial(7), antifungal, and antioxidant(8) activities. These facts prompted us to undertake a biological investigation of semipolar fraction of *A. kopetdaghensis* contains both class of sesquiterpenes and flavones in combination with chloroquine to assess their synergistic antimalarial activity against *Plasmodium berghei* in murine mice.

4- As usually sesquiterpene lactones are responsible for antimalaria effect, why the extract standardized based on flavonoids?

Response: Both methoxylated flavones and sesquiterpene lactones are responsible for observed effects. There is also good reference from flavonoids isolated from Artemisia annua with antimalarial effects. Currently, we have also conducted a recent study on a purified flavone from artemisia species with remarkable antimalarial effects, which is preparing to be published elsewhere.

However, we have also analyzed the sesquiterpene lactone contents simultaneously which is added to the text.

- Liu KC-SC, Yang S-L, Roberts M, Elford B, Phillipson J. Antimalarial activity of Artemisia annua flavonoids from whole plants and cell cultures. Plant Cell Reports. 1992;11:637-40.
- Rudrapal M, Chetia D. Plant flavonoids as potential source of future antimalarial leads. Systematic Reviews in Pharmacy. 2017;8(1):13-8.

The following response was added to the text in the section of standardization.

"Both methoxylated flavones and sesquiterpene lactones are responsible for observed effects. Therefore, the total flavonoid content (TFC) of the semipolar extract was analyzed by the complexation method after some modifications(1, 9)."

5- In results section has mentioned " The plant extract was simultaneously analyzed in phytochemistry lab to identify the STLs previously reported" and referred to previous study. it is recommended to mention it in method section if it has done for this study too.

Response: It was added briefly as follows in method section:

Phytochemical analysis

Briefly, *semipolar extract* filtered on a C-18 filled-sintered glass funnel using methanol:water (70:30) and chromatographed on silica gel column washed out using hexanes: Ethyl acetate in gradient order (97:3 -> 30:70; Fr.1–Fr.14). Based on ^{1}H-NMR experiment fractions contained sesquiterpene lactone signals were selected and more purified by recrystallization or HPLC on YMC silica column (chloroform: methanol; 90:10) to yield pure compounds as previously reported elsewhere (2).

6- In the discussion , there is no mention of the presence of possible compounds in the selected extract, considering the effectiveness of the results.

Response: The following phrase was added at the end of the discussion.

"Based on phytochemical analysis on the extract, *it contained Eudesmane SLs including* persianolide A (**1**), 4-epi-persianolide A (**2**), 3α,4-epoxypersianolide A (**3**), 11-epi-artapshin (**4**), 1β,8α-dihydroxy-11α,13-dihydrobalchanin (**5**), 1β-hydroxy-11-epi-colartin (**6**), as well as methoxylated flavones (1, 2), which are responsible for the observed effect. These compounds could be isolated in large quantities and evaluated in

their pure state as new lead candidates for antimalarial treatment along with standard drugs. ”

Associate Editor's Comments:

Associate Editor 1:
Dear Editor-in-chief This article, in the decision of reviewers, needs to be rewritten.

Response: The manuscript was checked. All the comments were addressed according to the referee's team point by point and highlighted in the text.

2 Revision 1

1 comment · 30 Jul 2024

Referee 1 Reviewer

ACCEPT 30 Jul 2024

Corrections have been made based on my previous comments.