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OPEN PEER REVIEW

Effect of *Alhagi maurorum* Hydroalcoholic Extract on Preventing Secondary Hydatid Cysts in BALB/c Mice

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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.

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1 Original submission

2 comments · 4 Mar 2023 – 7 Apr 2023

Somayeh Mirzavand Reviewer

MAJOR REVISION 4 Mar 2023

The manuscript is informative but needs major revision.
1. Most of the article's references are out of date. Please use more up-to-date references (2018 or later) and also arrange the references according to the journal's format.
2. Please check the manuscript; some words, such as "briefly" in line 105 and "Furthermore" in line 140, were repeated twice.
3. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.
4. How long after the mice were infected did you start the treatment?
5. Did secondary cysts develop in the control group?
6. What is your justification for the effect of this plant in preventing secondary cysts?
7. Did you have any missing values in your study? If so, please describe your strategy for solving this problem. This is critical for controlling nonresponse bias. Please mention it.
8. Please mention the length of the treatment period.
9. Please mention the limitations and strong points of your study.
10. The means and SDs should be presented in the tables, and it would be better to remove the sum.
11. In the tables, remove the group numbers and instead write the names of the different groups.

12. Significant or nonsignificant? Please present the mean (SD) or frequency (%) of each response variable according to each independent variable in a table. The test statistic and p-value must also be included in the table.
13. In bar charts, please clarify whether the error bars represent SE or SD.
14. The references section should be corrected according to the instructions for the authors and edited to include DOI numbers.

Editor-in-Chief

MAJOR REVISION 7 Apr 2023

Dear Authors,
Before proceeding further, you need to make major changes to the manuscript. Please revise the manuscript according to the provided comments and upload a point-by-point response to the comments along with the revised manuscript. Highlight any changes.

Best regards,
JJNPP EIC

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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.

Manuscript Number: 135321

Response to Reviewers

Editorial Office, Jundishapur Journal of Natural Pharmaceutical Products

On behalf of the authors of the of the manuscript entitled **"**Evaluation of the effect of Hydroalcohol *Alhagi maurorum* extract in the prevention of secondary hydatid cysts in BALB/c Mice**".** I would like to appreciate you and your team for reviewing the manuscript and giving an opportunity to submit the revised version for possible publication in the Journal of **Jundishapur Journal of Natural Pharmaceutical Products**. Most of the reviewers' comments are incorporated in the revised manuscript. We have revised the manuscript according to the suggestions and highlighted the textual changes in yellow. A reply to the specific comments raised by the reviewers is mentioned below.

Reviewer Comments:

Q1. Most of article references are out of date. Please use more up to date references (2018 or upper) and also arrange references according to journal format."

R: Thanks for critically reading and valuable comments of respected reviewer. The references were updated and arranged according to the journal format.

Q2. Please check the manuscript, some words like briefly in line 105 and Furthermore in line 140 were twice repeated.

R: Based on your valuable comments, corrected.

Q3. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.

R: Based on your valuable comments. This is an experimental study and the number of 28 mice was determined by a statistical consultant and also according to previous studies. The number of mice in each experimental group is mentioned in the method section.

Q4. How long after the mice infection did you start the treatment?

R: Thank you, based on your valuable comments. BALB/c mice were inoculated intraperitoneally with viable protoscoleces. One month after the infection, the infected mice were randomly divided into 4 groups. Revisions have been made and highlighted.

Q5. Were secondary cysts developed in the control group?

R: Thank you, yes, all 4 groups of mice (control, albendarol, *Alhagi maurorum* extract 500 and 250 mL/L) were infected with protoscoleces and after treatment, it was seen that the control group that did not receive treatment had the highest number of cysts. Highlighted to the results section.

Q6. What is your justification for the effect of this plant in preventing secondary cysts?

R: Thank you for your comment valuable. Research on *Alhagi maurorum* indicates that it contains sterols and fatty acids, alkaloids, and flavonoids. Since plants containing flavonoids have anti-protoscoleces effects. In addition, previous studies have shown that herbal extracts can induce the apoptotic pathway and because apoptosis is a process of cellular self-destruction, it provides a convenient way to eliminate parasitic agents, such as *Echinococcus* protoscoleces. Therefore, for the reasons mentioned, in this study, for the first time, the effect of the hydroalcoholic *Alhagi maurorum* extract against secondary hydatid cysts in BALB/c mice was investigated.

Highlighted to the disscution section.

Q7. Did you have any missing value in your study? If it was, please write what was your strategy for solving this problem. It is critical points for control non response bias. please mention it.

R: Thank you. This disease is not fatal in mice. One month after infecting the washes with live protoscoleces, we started the treatment for six weeks, all the mice survived until the end of the treatment period and we did not lose any of the mice.

Q8. Please mention the length of the treatment period.

R: Thank you, corrected and highlighted to the method section. The length of treatment was 6 weeks.

Q9. Please mention the limitations and strong points of your study.

R: Thank you for your comment valuable. The limitations and strengths of our study were added to the manuscript. In this study, for the first time, the effect of the hydroalcoholic *Alhagi maurorum* extract against secondary hydatid cysts in BALB/c mice was investigated. In our study, it was not precisely determined which compound of the *Alhagi maurorum* plant has an anti-protoscoleces effect, and more studies are needed to accurately judge the effect of this plant on hydatid cysts.

Q10. The means and SD should be presented in the tables and it is better to sum be removed.

R: Thank you for your valuable comment, the data in the table was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test and P <0.05 was considered statistically significant and the significant difference between the groups was presented in the form of a graph in Fig. 3. In order to better understand the results of the study, the table was removed and the results were presented in the form of a graph.

Q11. In the tables, remove the group numbers and instead write the names of different groups.

R: Based on your valuable comments, in Table 1, the numbers of the groups were removed and the names of the groups were replaced instead.

Q12. Significant or non-significant? Please write (Mean (SD) or frequency (%)) of each response variable according to each independent variable in a table, also the test statistic and p value must come to the table

R: Thank you. In order to better understand the results of the study, the table was removed and the results were presented in the form of a graph. The data in the table was analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test and P <0.05 was considered statistically significant and the significant difference between the groups was presented in the form of a graph in Fig. 3.

Q13. In bar charts, please clarify that whether error bars are SE or SD

R: Thank you, errors *bars* show SD in different groups. Fig. 3 caption was corrected.

Q14. The references section should be corrected according to the instructions for the authors and supplemented with DOI numbers edited.

R: Thank you for pointing this out. The references were updated and arranged according to the journal format.

With the best regards, sincerely yours

Dr. Hakim Azizi

Corresponding Author

2 Revision 1

1 comment · 7 Jun 2023

Referee 1 Reviewer

MINOR REVISION 7 Jun 2023

Dear authors,
Thank you for revising the manuscript according to the reviewer's comments, but there are still some problems that need to be corrected.

1. Please include your answer to question 7 in the manuscript.
2. It is necessary to report the voucher number of the plant used. If the Botanical Section of Zabol University of Medical Sciences identified the plant, ask them to give you the voucher number.
3. How much extract was injected into the mice in groups 3 and 4? You reported only the concentration of the extracts (mg/ml), but it is necessary to mention the amount of extract used, as with albendazole (mg/kg).
4. "g" is the correct abbreviation for gram instead of "gr". Please correct "gr" in the manuscript.
5. It is necessary to write the full term for "PBS" at its first mention.
6. Hydroalcohol extract is more common than hydroalcoholic extract; please use the extract type consistently throughout the manuscript.
7. In sentences such as "In the first group, each mouse was experimentally infected with an oral injection of PBS", "infected" is not the correct verb. Please change it.
8. The sentence "The results of this study have demonstrated that treating infected mice with hydroalcoholic Alhagi maurorum extract efficiency in the treatment of hydatid cysts" is grammatically incorrect.
9. Finally, there are few comments in the attached file; I hope they can help you improve the manuscript.

Best regards

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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.

Manuscript Number: 135321

Response to Reviewers

Editorial Office, Jundishapur Journal of Natural Pharmaceutical Products

On behalf of the authors of the of the manuscript entitled **"**Evaluation of the effect of hydroalcohol *Alhagi maurorum* extract in the prevention of secondary hydatid cysts in BALB/c Mice**".** I would like to appreciate you and your team for reviewing the manuscript and giving an opportunity to submit the revised version for possible publication in the Journal of **Jundishapur Journal of Natural Pharmaceutical Products**. Most of the reviewers' comments are incorporated in the revised manuscript. We have revised the manuscript according to the suggestions and highlighted the textual changes in green. A reply to the specific comments raised by the reviewers is mentioned below.

Reviewer Comments:

Q1. Please mention your answer to question 7 in the manuscript

- Did you have any missing value in your study? If it was, please write what was your strategy for solving this problem. It is critical points for control non response bias. please mention it.

R: Thanks for critically reading and valuable comments of respected reviewer. We found no missing value in this study.

Q2. It is necessary to report the voucher number of the used plant. If the Botanical Section of Zabol University of Medical Sciences identified the plant, ask them to give you the voucher number.

R: Based on your valuable comments, corrected, (Herbarium number 472).

Q3. How much extract were injected to the mice in group 3 and 4? You just reported the concentration of extracts (mg/ml) but it is required to mention the amount of used extract like albendazole (mg/kg).

R: Based on your valuable comments. Corrected.

Q4 "g" is the correct abbreviation of gram instead of "gr". Please correct "gr" in manuscript.

R: Thank you, based on your valuable comments. Corrected.

Q5. It needs to write the full term of "PBS" for the first time.?

R: Thank you, corrected.

Q6. Hydroalcohol extract is more common than the hydroalcoholic extract, please write uniform the extract type throughout the manuscript?

R: Thank you for your comment valuable. Corrected.

Q7. In the sentences such as "In the first group, each mouse was experimentally infected with an oral injection of PBS" the infected is not the correct verb. Please change it.

R: Thank you. Corrected and highlighted in treatment of mice.

Q8. The sentence "The results of this study have demonstrated that treating infected mice with hydroalcoholic Alhagi maurorum extract efficiency in the treatment of hydatid cysts" is grammatically mistake.

R: Thank you, corrected and highlighted.

Q9. Finally, there are few comments in the attached file, hope can help you to improve the manuscript.

R: Thank you for your comment valuable. All comments have been corrected.

With the best regards, sincerely yours
Dr. Hakim Azizi
Corresponding Author

3 Revision 2

1 comment · 24 Jun 2023

Referee 1 Reviewer

ACCEPT 24 Jun 2023

Dear authors,
Thank you for your response to the comments of reviewers. The manuscript entitled “Evaluation of the effect of hydroalcohol Alhagi maurorum extract in the prevention of secondary hydatid cysts in BALB/c Mice” is accepted for publication in Jundishapur Journal of Natural Pharmaceutical Products.
With respect