Iranian Journal of Pharmaceutical Research
Volume 22 · Issue 1 · e140323

OPEN PEER REVIEW

Antifatigue Effects of the Aqueous Extracts of Myrtle Berries, Apple and Clove: An Animal Study

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SUBMITTED	REVISED	ACCEPTED	DOI
27 Aug 2023	13 Sep 2023	23 Sep 2023	10.5812/ijpr-140323

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

1 comment · 30 Aug 2023

Referee 1 Reviewer

MINOR REVISION 30 Aug 2023

-Please check the entire manuscript for English, including grammar, spelling, italic formatting, and ….
(for example, line 101: "The rat only had access to water the previous night as they
had to fast"): use "the rats".
-The keywords don't mention all the plants; please add the other two plants too.
- In the abstract, it's better to mention how many rats were evaluated in the five groups.
- Why were aqueous extracts used for this study? Hydroalcoholic extracts may usually contain more active ingredients.
- On what basis were the doses of the extract selected for the tests?
- Why was Gallic acid tested as an HPLC standard? Myrtle contains other, more important active ingredients.
-In the methods section, some parts have no references, for example, the extraction method (How are the authors sure that boiling the extracts or drying at 70°C does not degrade the active ingredients?) and some pharmacological tests (Open field test). Please check and modify.
- The authors have concluded that "These observed effects can be due to phenolic compound such as gallic acid in myrtle extract". Since clove and apple juice also contain phenolic compounds, it's better to explain further why those extracts did not show similar results.
- Also, based on this conclusion, it would have been better to determine the total phenol content and phenolic compounds in the extracts to help improve the comparison, discussion, and conclusion, which could then be written more completely.
- It seems that there is no need for many references in some parts. For example, the first sentence of the discussion, which is a general explanation, does not need to have 5 references.
- Figure captions in the appendix (end of the manuscript) are not numbered.

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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.

Dear Dr. Soraya Shahhosseini,

Thank you for giving us the opportunity to submit a revised draft of our manuscript titled "Antifatigue effects of aqueous extracts of myrtle berries, apple and clove using animal models" to "*Iranian Journal of Pharmaceutical Research*". We appreciate the time and effort that reviewers have dedicated to providing their valuable feedback on our manuscript. We have highlighted the changes within the manuscript. The reviewers' questions have been answered separately for each reviewer in the following.

Best regards.

Reviewer 1
Please check all manuscript for English; grammar, dictation, writing italic and …. *(for example line 101: The rat only had access to water the previous night as they had to fast) :the rats*
The sentences were rechecked for spelling and grammar, and adjustments were made. Corrections in writing were not highlighted.
In the keywords doesn't mention all plants, please add two other plants too.
Based on the reviewer's comment, the names of two other plants were added to keywords.
In abstract, it's better to mention how many rats in five group evaluated.
Based on the reviewer's comment, it was added to abstract.
Why aqueous extracts used for this study? as usually hydroalcoholic extracts maybe contain more active ingredients.
The type of extract was selected based on Iranian traditional medicine references. They have used aqueous extract of these three plants.
On what basis were the doses of the extract selected for the tests?
Animal dose was determined based on the doses included in traditional medicine references.
Why Gallic acid tested for HPLC standards? The myrtle contains more important other active ingredients.
The myrtle berries has a lot of phenolic compounds, thus we employed gallic acid as marker compound to standardize it. Considering that oxidative stress and inflammatory factors are effective in the occurrence of fatigue, therefore compounds with antioxidant and anti-inflammatory effects can be effective in reducing fatigue. The phenolic compounds have antioxidant and anti-inflammatory effects which can be effective in the occurrence of anti-fatigue effects.
In method section some parts has not references, for example for method of extraction (How are the authors sure that boiling the extracts or drying at 70°C does not degrade the active ingredients?) and some pharmacological tests (Open field test), please check and modify.

The references were added.
the authors have concluded that;"These observed effects can be due to phenolic compound such as gallic acid in myrtle extract". Since the clove and apple juice also contain phenolic compounds, it's better to explain more why those extracts had not shown similar results.
As we have stated in the discussion of the article, the effects we have observed for myrtle berries are probably related to the phenolic compounds, flavonoid and polysaccharides present in it. This issue is a possibility. One of the goals of our future study is to isolate the compounds of the aqueous extract of the myrtle berries and investigate the pharmacological effects of these compounds. In this manuscript, we used gallic acid just for standardization of aqueous extract of myrtle berries. The results of weight-loaded forced swimming test showed that two other plants were also effective, but not as much as myrtle. The aqueous apple extract increased the swimming time, or aqueous extract of clove decreased the LDH level, and both (apple and clove) increased the glucose level but it wasn't significant. The effectiveness of clove essential oil in lowering LDH levels has demonstrated previously, which has been associated with the presence of eugenol and the antioxidant activity of essential oil. The primary component of clove oil is eugenol. It is highly soluble in organic solvents and slightly soluble in water. Therefore, the aqueous extract of clove contains a small amounts of eugenol. As a result, the essential oil of clove probably is more efficient at lowering LDH levels than the aqueous extract of cloves.
Also based on this conclusion, it was better to determine total phenol and phenolic compounds in the extracts that helps to better comparison, discussion and conclusion (that can write more complete).
Given that the chemical compound responsible for the anti-fatigue effects of myrtle berries was unknown, a standardized approach using a marker compound in myrtle berries and a more precise method, the HPLC method rather than the total phenol determination method, was used. For standardization of medicinal plants, active compounds or marker compounds are quantified. If determination of active compounds does not be possible, a marker compound will be quantified. In case of apple, an active or marker component is not clear. In clove, eugenol is considered as a marker for quality control of the plant. Therefore although apple and clove contain phenolic compounds, but these components are not considered as active or marker of these plants.
It seems that there is no need for many references in some parts. For example, the first sentence of discussion, which is a general explanation, does not need to have 5 references.
Based on the reviewer's comment, it was modified and additional references were removed.
Figures caption in the in the appendix (end of the manuscript) are not numbered.

Thanks to your attention, the number of figure was added for each figure.
Reviewer 2
Please add the graphical abstract
Thank you for your considerations and attention. Based on reviewer's comment, the graphical abstract was added.
Organized abstract in the result sections (line 20-27); the effect of the treatment in the behavioral test are not well reported.
Based on reviewer's comment, the corrections were made.
Please change "acute toxicity" to "acute oral toxicity test" in line 98.
Based on reviewer's comment, the corrections were made.
The order of uploaded figures should be corrected.
Thanks to your attention, the order of uploaded figures have been checked and corrected.
Please add the number of figures for all the uploaded figures. For example, add figure 1 or 2 or 3 in the legends.
Thanks to your attention, the number of figure was added for each figure.
The names of the behavioral tests are missing from the legends of figures 2 and 4.
Thanks to your attention, the names of the tests were added for each figure.

2 Revision 1

0 comments

No public comments in this round.