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OPEN PEER REVIEW

Evaluating the Effectiveness of Aqueous Extract of Persian Oak (*Quercus castaneifolia* C.A.Mey.) Fruit Hull on Dermal Wound Healing in the Rat Model

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1 Original submission

1 comment · 23 May 2022

Editor-in-Chief

MAJOR REVISION 23 May 2022

Dear Authors,

Your manuscript has been scrutinized by three independent reviewers, and they have raised significant issues that should be addressed before a decision can be made. Please provide a point-by-point answer or rebuttal to the reviewers' comments with the revised manuscript and highlight changes.

Kind 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.

Dear editor

Thanks to the reviewers for taking the time to review our article and for their helpful comments. We appreciate your feedback, and it will help us improve the quality of our article. Since all the corrections have been made, we hope the manuscript will now be accepted without any further changes. We look forward to hearing from you regarding our submission.

Reviewer 1:

1. Abstract: Please revise the result section and improve it by addition of quantitative measures. A quantitative criteria related to the results was added to the abstract.

2. Background: Line 38: Please delete the additional "enter" that interrupted the line. The text was corrected.

3. Method: Statistical analysis section was missed. Please correct it. Section 2.9 is entitled "Statistical Analysis" in the article Lines 151-154.

4. Discussion: Please add more justification for the results obtained in this study and improve discussion section by comparing the results with other studies. The results were compared, and the text was corrected.

Reviewer 2:

1. It is highly suggested that authors expanded the Introduction section. An explanation was added to the introduction section that has been highlighted.

2. In material and methods, the authors must write the reference or references. References 16-23 are written for sections 2.2, 2.3, 2.5, 2.6 and 2.7. Another reference (No. 21) was added to the tensile strength test (Section 2.7)

3. Discussion is poorly written. It is highly suggested that authors expanded the discussion section and explain about the purpose of selecting the soluble form (due to the fact that

high humidity is not suitable for wound healing) and plant extracted compounds in this part. The purpose of selecting the aqueous extract of Jaft was to evaluate the effectiveness of this extract as a wound washing solution and its effect on the wound healing process. Most previous research has used oak or Mazo extract in alcohol or ointment bases and applied it to wounds or burn wounds. In this study, aqueous extract spray was used on the wound area. The description added to the discussion is highlighted.

4. Abbreviations should be introduced in the text for the first time that they present (VEGF and PDGF). These terms were introduced in the abstract and in the article. (Added text was highlighted)

5. What animal was used in this research? It is mentioned in the title of using rat while in line 104 it is mentioned in mice. The animal used in this study was male Wistar rat, and its name was corrected.

6. The results of days 10 and 12 are not mentioned either in the figures or in the text. The results related to the percentage of wound healing on days 10 and 12 are shown in Figure 2. Skin's tensile strength, growth factors, and histology were performed only at the end of the treatment period.

7. For the claims mentioned in lines 229 and 253, please mention the scientific reasons. (A high concentration of tannins in Jaft extract can cause cytotoxicity and thus delay the healing process (229) and The restorative effect of this extract is due to the increase in angiogenesis (253)) In similar studies (reference numbers 24 and 26) on Mazo extract and tannic acid, it was mentioned that the reason for the lack of appropriate wound healing in high concentrations of the extract was due to the high concentration of tannin, which probably has a cytotoxic effect. We also observed in our research that a high concentration of aqueous extract of Jaft prevents proper wound healing.

The study by Bao et al. (Reference 29) suggested that high vascular endothelial growth factor was directly related to angiogenesis. In similar studies conducted by Hemmati et al. (Reference 30) and Shabanian et al. (Reference 31), it was found that oak extract has a positive effect on angiogenesis and causes new vascular buds and capillary formation. As a result, it increases

blood flow to the damaged tissue and accelerates tissue repair. The same effect was observed in the present study.

8. Avoid repeating the presented results in the discussion section. Duplicate content was removed from the discussion.

9. Please indicate the amount of fibroblasts and granulation or other related symptoms on the pathology images with an arrow. The restored tissue components were marked on the photos with an arrow.

Reviewer 3:

1. Regarding the conditions of keeping animals, considering that the conditions have been controlled, keeping animals in an environment with a humidity of about 60% is not suitable and will definitely affect the quality of wound healing. This was a misspelling that was corrected in the text.

2. When the placenta powder is soaked in boiling distilled water for 24 hours, all its active ingredients are destroyed. I do apologize that this part was not clear in the original manuscript. I should have explained that The Jaft powder was poured into boiled water and then placed on a shaker for 24 hours, but no heat was given during this time. I have revised the contents of this part.

3. Can the volume of 10 liters of distilled water used to extract the extract be separated at a temperature of 40 degrees because after a long time at a not too high temperature there is a possibility of spoilage and mold of the extract. Extraction was performed several times, and each time the aqueous extract was placed in a vacuum oven until completely dry. The drying operation is performed faster in a vacuum without giving the extract a high temperature.

4. Using a temperature of 40 degrees for drying is to ensure that the active ingredients in the extract are not destroyed, while in the previous stage, the extract was boiling for 24 hours so it does not seem logical. In the first stage, the Jaft powder was poured into boiled

water, but then it was not heated, and after soaking for 24 hours (on a shaker), it was filtered. The Jaft powder was not boiled but poured into boiled water.

5. In the Materials and methods, instead of Skin wound induction, use the term Skin wound creation. The title is corrected in the text.

6. In the field of Skin wound induction, the type of animal used is mice. Finally, the authors specify whether they used rats or mice. The animal used in this study was male Wistar rat, and its name was corrected.

7. Given that two wounds are created in each animal; How the sample size was calculated? Describe the statistical calculations of the sample size in this study. The number of samples was calculated based on reference number 24 and 31

8. The quality of histological images is not good, especially in the case of Mason trichrome staining; On each image, specify the different layers of skin and the characteristics of skin tissue repair. Layers of skin and some restored tissue components were marked with arrows.

9. In the results of the study in the section related to "Measurement of tensile strength", according to the compliance of the results of the present study with the following study, refer to it. This reference is added.

2 Revision 1

0 comments

No public comments in this round.