OPEN PEER REVIEW

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Peer Review Report for "*Echeveria subrigida* Leaf Extracts Standardized for Isorhamnetin-3-O-Glucoside Show Antidiabetic Effects via the PI3K/Akt Pathway in Rats"

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Review Timeline:

Submit Date:	4 Dec 2024
Revised Date:	18 Jan 2025
Accepted Date:	29 Jan 2025

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Esmaeil Mohammadian : Reviewer

10 Dec 2024

Dear Editor
Have a nice day.
The author investigated Antidiabetic effects of a standardized extract of Echeveria subrigida leaves in diabetic rats via activation of hepatic PI3K/Akt pathway.
I would suggest reconsidering the manuscript for publication only after the following major revision.
1. Please mention the commercial source of all the materials and the model of the

instrument used.
2. Please report mean± SD for all values (with two decimal places).
3. Please revise the manuscript for lingual quality and typo mistakes.
4. Please check all abbreviations.
5. Please specify the positive control.
6. The title needs to change.
7. Please rewrite the introduction section based on the title.
8. A complete conclusion section should be written.
9. Please mention the obtained quantitative results in the abstract section.
10. Please report the HPLC graph, retention time, and purity.

REFEREE : Reviewer

21 Dec 2024

There are some minor English grammar mistakes that need to be addressed.

Can the authors explain the novelty of their work. In the introduction there are many references that explain the IC50 and type of extract used for antidiabetic effects.

Please state the abbreviations clearly the first time they are used
Methanol Extract (MEEs)

mg/kg b.w. : when you state kg it means body weight.
also abbreviations should be presented the first time then used in the text. body weight (b.w.)

are the compositions of methanolic extracts and ethanolic extracts simillar? why have the authors used isorhamnetin as the same indicator for both extracts? If isorhamnetin is present in both extracts, why use both methanolic and ethanolic extracts rather than only use the extract with the highest isorhamnetin ?

in the oral glucose tolerance test how was glucose was measured after 30, 60, and 120 min? by sacrificing or drawing blood? if drawing blood where was the blood drawn from?
how many times was blood drawn from one single subject?

what do the authors suggest are the other compounds that induce synergistic glycemic effects?

in the introduction it would be beneficial to explain the connection between leptin and DM.

Overall it is a very valuable research, however in some parts the data explanation and sentences are too complex to follow while the scientific writing in some parts can be improved.

good luck

REFEREE : Reviewer

24 Dec 2024

This manuscript investigated the antidiabetic effects of a standardized extract of Echeveria subrigida leaves in a diabetic animal model.

The manuscript represents interesting results. However, they are not well presented at all.

Therefore, I highly suggest revising the manuscript with regard to English language and also discussion of the results.

There are also a few questions which should be addressed:

1. Since the authors measured PPARα expression, why was not pioglitazone considered as an antidiabetic agent?
2. No p values are reported in the tables which should be added.
3. Were all data distributed normally?
4. How do authors justify the effect of ISO on blood glucose levels?
5. How were the results of each extract comparable with metformin?

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

ANSWERS TO REVIEWERS

Journal: Jundishapur Journal of Natural Pharmaceutical Products

Manuscript ID: 158548

Manuscript Title: Antidiabetic effects of a standardized extract of *Echeveria subrigida* leaves in diabetic rats via activation of hepatic PI3K/Akt pathway

Modifications were yellow highlighted in the manuscript.

Reviewer 1

1. Please mention the commercial source of all the materials and the model of the instrument used.

   Answer: The requested information was included in the manuscript.

2. Please report mean± SD for all values (with two decimal places).

   Answer: The manuscript was corrected according to the recommendation.

3. Please revise the manuscript for lingual quality and typo mistakes.

   Answer: The manuscript was revised and changes were introduced

4. Please check all abbreviations.

   Answer: Abbreviations were reviewed, and changes were included in the manuscript.

5. Please specify the positive control.

   Answer: In the experimental design for the antidiabetic activity, metformin was used as a positive control (please see section 3.5). For the assay of protein expression, GAPDH was used as a marker of constitutive expression (please see section 3.10).

6. The title needs to change.

   Answer: The title was modified to "Echeveria subrigida leaf extracts standardized for isorhamnetin-3-O-glucoside show antidiabetic effects via the PI3K/Akt pathway in rats".

7. Please rewrite the introduction section based on the title.

   Answer: The introduction was reviewed, and it is aligned with the title.

8. A complete conclusion section should be written.

   Answer: The conclusions were included in a separated section.

9. Please mention the obtained quantitative results in the abstract section.

   Answer: The abstract was modified to meet the recommendation.

10. Please report the HPLC graph, retention time, and purity.

    Answer: Figure 1 was included and the following information is included in section 4.1. "… The peak purities of Q3G (999.36) and I3G (998.69) showed a good separation (> 990), while the peak of tannins resulted in a mixture of compounds with a value of 977.08 (Figure 1)."

Reviewer 2

1. *The use of streptozotocin-induced diabetic rats is a suitable model for evaluating antidiabetic properties. However,* the methodology could be improved by elaborating on the dosing regimens for the extracts and controls (isorhamnetin and metformin), as well as the date of plant collection would enhance reproducibility.

   Answer: The experimental design to evaluate the antidiabetic effect is shown in section 3.5., appearing as follows:

   "… Six groups of animals (n = 6) were formed: HC = healthy control; DC = diabetic control; MET = diabetic + metformin treatment (500 mg/kg b.w.); ISO = diabetic + isorhamnetin (1.9 mg/kg b.w.); EEEs = diabetic + Echeveria subrigida ethanolic extract (400 mg/kg b.w.); and MEEs = diabetic + Echeveria subrigida methanolic extract (284.7 mg/kg b.w.). The employed doses of ISO, EEEs, and MEEs were adjusted to provide the same quantity of isorhamnetin. Oral treatments were administered once a day (17:30 h) for 30 days."

   The date of plant collection was included in section 3.2., November 2019.

2. The terminology used throughout is appropriate for a scientific audience. However, consider defining acronyms (e.g., MEEs for Methanol Extracts, EEEs for Ethanol Extracts) upon first use for clarity, especially for readers less familiar with the subject.

Answer: The manuscript was modified to define all acronyms the first time mentioned.

3. Specify the euthanasia method and include details about the euthanasia method used to ensure compliance with ethical standards.

Answer: The section 3.5. was modified as follows: "… rats were euthanized following the Mexican Official Norm NOM-062-ZOO-1999. The animal was anesthetized with ether, and blood was collected by cardiac puncture in polystyrene tubes without anticoagulant (BD Vacutainer®). Subsequently, manual cervical dislocation was performed…"

4. Indicate the number of samples analyzed for MCP-1 and leptin and others, and whether the assays were performed in duplicate or triplicate to ensure reliability.

Answer: Information was included in Section 3.7.: "… Three biological replicates were measured per duplicate: rats in each experimental group were randomly allocated into groups of two rats, and their sera were mixed to measure the MCP-1 and leptin levels."

Information was added to section 3.8.: "… The analysis was carried out by triplicate as indicated in section 3.7."

In the section 3.12 was added: "… Evaluations were carried out at least in triplicate…"

5. Details on PCR conditions, include information about the specific PCR conditions (e.g., annealing temperature, cycle numbers) used during the thermal cycling to enhance reproducibility.

Answer: The employed PCR parameters were included in the section 3.11. as follows: "… A 2720 thermal cycler (Applied Biosystems™, Foster City, CA, USA) was used, and the running conditions included initial denaturation at 95˚C for 30 s, followed by 35 cycles at 94˚C for 15 s, 60˚C for 35 s, and 72˚C for 40 s."

PCR parameters:

Cycle step	Temp.	Time	Cycles
Initial desnaturation	95°C	30 sec.	1

Desnaturation	94°C	15 sec.	35
Annealing	60 °C	35 sec.	
Extension	72°C	40 sec.	
Final extension	72°C	5 min.	1

6. Mention the number of biological replicates used for the gene expression analysis to provide insight into the statistical power of the results.

   Answer: Information was added to section 3.10.: "… The protein expression was measured with the pooled livers of each group, three extractions were carried out and analyzed by duplicate…"

   In section 3.11., it was added: "The mRNA expression was measured with the pooled livers of each group, three extractions were carried out and analyzed by duplicate.…"

   In section 3.12 was added, "… Evaluations were carried out at least in triplicate..."

Reviewer 3

1. The manuscript represents interesting results. However, they are not well presented at all.

   Answer: The manuscript was reviewed and modified.

2. Therefore, I highly suggest revising the manuscript with regard to English language and also discussion of the results.

   Answer: The English was reviewed and changes were done.

There are also a few questions which should be addressed:

3. Since the authors measured PPARα expression, why was not pioglitazone considered as an antidiabetic agent?

   Answer: Many papers have reported that pioglitazone is a PPARγ agonist. Sakamoto et al. (2000) showed that pioglitazone was a strong activator of hPPARγ1 but a weak one of hPPARα. In this research, it was measured the liver isoform, where predominate the PPARα

isotype (Abbot 2009). Moreover, the objective was to look for the possible interaction between the PPARα expression and the activation of the AMPK pathway, a mechanism of action that is reported for some antidiabetic drugs (Lee et al. 2006; Lee et al. 2010; JinJin et al. 2014; Jin et al. 2017).

- Junichi Sakamoto, Hiroyuki Kimura, Shinji Moriyama, Hiroyuki Odaka, Yu Momose, Yasuo Sugiyama, Hidekazu Sawada, Activation of Human Peroxisome Proliferator-Activated Receptor (PPAR) Subtypes by Pioglitazone, Biochemical and Biophysical Research Communications, Volume 278, Issue 3, 2000, Pages 704-711, ISSN 0006-291X, https://doi.org/10.1006/bbrc.2000.3868.
- Abbott, BD (2009). Revisión de la expresión de los receptores activados por el proliferador de peroxisomas alfa (PPARα), beta (PPARβ) y gamma (PPARγ) en el desarrollo de roedores y humanos. Toxicología reproductiva, 27(3-4), 246–257. doi:10.1016/j.reprotox.2008.10.00.
- Woo Je Lee, Mina Kim, Hye-Sun Park, Hyoun Sik Kim, Min Jae Jeon, Ki Sook Oh, Eun Hee Koh, Jong Chul Won, Min-Seon Kim, Goo Taeg Oh, Michung Yoon, Ki-Up Lee, Joong-Yeol Park, AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARα and PGC-1, Biochemical and Biophysical Research Communications, Volume 340, Issue 1, 2006, Pages 291-295, ISSN 0006-291X, https://doi.org/10.1016/j.bbrc.2005.12.011.
- Lee, Woo Hyung, Kim, Sang Geon, AMPK-Dependent Metabolic Regulation by PPAR Agonists, *PPAR Research*, 2010, 549101, 10 pages, 2010. https://doi.org/10.1155/2010/549101
- JinJin Yin, YanQin Luo, HouLiang Deng, ShuMin Qin, WaiJiao Tang, Lu Zeng, BenJie Zhou, Hugan Qingzhi medication ameliorates hepatic steatosis by activating AMPK and PPARα pathways in L02 cells and HepG2 cells, Journal of Ethnopharmacology, Volume 154, Issue 1, 2014, Pages 229-239, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2014.04.011.
- Jin Huang, Saeromi Kang, Soo-Jin Park, Dong-Soon Im, Apelin protects against liver X receptor-mediated steatosis through AMPK and PPARα in human and mouse hepatocytes, Cellular Signalling, Volume 39, 2017, Pages 84-94, ISSN 0898-6568, https://doi.org/10.1016/j.cellsig.2017.08.003.

4. No p values are reported in the tables which should be added.

   Answer: The means were contrasted by the Fisher test, which employs one LSD value for each parameter. The tables showed the mean ± SD, and in the footnote of the tables, it is indicated that statistical differences were established when $P < 0.05$.

5. Were all data distributed normally?

   Answer: Normality was corroborated by the Shapiro-Wilk test. Please, see the statistical analysis section.

6. How do authors justify the effect of ISO on blood glucose levels?

Answer: It has been reported the antidiabetic effect of isorhamnetin (Matboli et al. 2021). This effect was not observed in this research because the isorhamnetin concentration employed was low, corresponding with the concentration found in the ethanolic (EEEs) and methanolic (MEEs) Echeveria extracts. Then, it was demonstrated that isorhamnetin is not the main responsible for the antidiabetic effect of the EEEs and MEEs.

- Matboli M, Saad M, Hasanin AH, L AS, Baher W, Bekhet MM, et al. New insight into the role of isorhamnetin as a regulator of insulin signaling pathway in type 2 diabetes mellitus rat model: Molecular and computational approach. *Biomed Pharmacother*. 2021;**135**:111176. [PubMed ID:33401224]. https://doi.org/10.1016/j.biopha.2020.111176.

7. How were the results of each extract comparable with metformin?

Answer: Globally, treatments with metformin and the methanolic extract of Echeveria subrigida (MEEs) showed similar and the best antidiabetic effects, followed by the ethanolic extract (EEEs), reaching statistical differences with the damage control ($P < 0.05$). However, metformin acts by the AMPK pathway, as expected by previously published data, whereas the Echeveria extracts act by the PI3K/Akt pathway.

Reviewer 4

1. There are some minor English grammar mistakes that need to be addressed.

Answer: English was reviewed, and some modifications were introduced.

2. Can the authors explain the novelty of their work. In the introduction there are many references that explain the IC50 and type of extract used for antidiabetic effects.

Answer: Echeveria subrigida is a plant native to Mexico and our research group has demonstrated its relevant biological activities as well as its chemical composition. Previous evaluations with this plant have been carried out in vitro or in vivo with normoglycemic animals. The antidiabetic effect/mechanism of the investigated extracts (ethanolic and methanolic) has not been previously demonstrated, both aspects were demonstrated in this research. Information that could be useful in the development of a preventive/therapeutical

antidiabetic product based on Echeveria subrigida. This could be useful for the sustainable use of this plant.

3. Please state the abbreviations clearly the first time they are used Methanol Extract (MEEs) mg/kg b.w. : when you state kg it means body weight. also abbreviations should be presented the first time then used in the text. body weight (b.w.)

   Answer: The abbreviations were defined the first time used, as suggested.

4. are the compositions of methanolic extracts and ethanolic extracts simillar? why have the authors used isorhamnetin as the same indicator for both extracts? If isorhamnetin is present in both extracts, why use both methanolic and ethanolic extracts rather than only use the extract with the highest isorhamnetin ?

   Answer: The ethanolic (EEEs) and methanolic extracts (MEEs) of Echeveria subrigida showed similar components, but their proportions are different (Figure 1). Specifically, the main difference is that tannin concentration was higher in the MEEs than in the EEEs. The lack of antidiabetic effect of isorhamnetin at the evaluated concentration suggests that the antidiabetic effect of the Echeveria extracts is due to the synergic effect of isorhamnetin, quercetin, and tannins. It must be emphasized that the quantities employed for both EEEs and MEEs were chosen to provide the same dose of isorhamnetin.

5. in the oral glucose tolerance test how was glucose was measured after 30, 60, and 120 min? by sacrificing or drawing blood? if drawing blood where was the blood drawn from? how many times was blood drawn from one single subject?

   Answer: The three glucose measurements were done in each rat of the group by sampling blood from the tail and employing an Accu-Chek instant instrument (please see sections 3.5 and 3.6).

6. what do the authors suggest are the other compounds that induce synergistic glycemic effects?

   Answer: In both Echeveria extracts (EEEs and MEEs), isorhamnetin, quercetin, and tannins are the main components. However, the tannin content of the MEEs is higher, corresponding with its better antidiabetic effect. Considering that the antidiabetic effect of the EEEs was also statistically significant and slightly lower than that of the MEEs, components in the MEEs and EEEs must be showing synergically antidiabetic effects.

7. in the introduction it would be beneficial to explain the connection between leptin and DM.

   Answer: The following information was added in the section 1: "… The leptin level is another parameter associated with obesity and diabetes, and this parameter is decreased in DM2 patients (7, 8)."

8. Overall it is a very valuable research, however in some parts the data explanation and sentences are too complex to follow while the scientific writing in some parts can be improved.

   Answer: Manuscript was reviewed and modified.

Associate Editor's Comments:

Associate Editor 1:

1. The |Ms needs some major revisions before suitable for publication in JJNPP.

   Answer: The manuscript was modified accordingly with the reviewers' comments.

Reviewer Assistant Comments:

These comments were generated by AI based on your manuscript. Please review them and consider incorporating the suggestions to enhance your article.

Based on the abstract provided, the study design appears to be an experimental study involving animal models. Therefore, the appropriate checklist for peer review would be the CONSORT checklist for Randomized Trial Studies, as it is often used for experimental studies, including those involving animal models.

6. Question of the checklist: Was the sample size and method of allocation to groups described?

- **Peer Review**: The abstract does not provide details on the sample size or the method of allocation to groups.

7. Question of the checklist: Were the statistical methods used to compare groups for primary outcomes described?

- **Peer Review**: The abstract does not mention the statistical methods used for data analysis.

8. Question of the checklist: Were the results for each group, including estimated effect size and precision, clearly presented?

- **Peer Review**: The results are presented, indicating the antidiabetic effects of the treatments, but the abstract lacks specific data on effect sizes and precision.

9. Question of the checklist: Were the limitations of the study discussed?

- **Peer Review**: The abstract does not discuss any limitations of the study.

Major Comments:

1. The abstract lacks details on the sample size and method of allocation to groups.

   Answer: Abstract was modified by adding the following: "… The diabetic rats were randomly allocated to the experimental groups (n = 6), parameters were measured at least in triplicate, and means were contrasted by the Fisher test (P < 0.05)…"

2. There is no mention of the statistical methods used for data analysis.

   Answer: Please, see the previous answer.

3. The abstract does not discuss any limitations of the study.

   Answer: Results of this study can not be assumed to be valid for humans. Then, future studies must consider clinical studies. This is not mentioned directly in the abstract, but the paper suggests that E. subrigida has the potential to be used in products to prevent or treat diabetes.

Minor Comments:

1. The abstract could benefit from including specific data on effect sizes and precision.

   Answer: Please, see the answer to question 1 in the previous section.

2. More detailed information on the interventions, such as dosage and duration, would enhance clarity.

   Answer: Authors consider that modified abstract contained the essential information.

OPEN PEER REVIEW

Revision (1)

Esmaeil Mohammadian : Reviewer — 23 Jan 2025

Dear Editor
Have a nice day.
The addressed issues and concerns have been effectively resolved. Consequently, the reviewer concurs to proceed with the publication of this paper in its current state.

REFEREE : Reviewer — 29 Jan 2025

The issues have been solved.