Reviewer’s report

Title: Alcohol extract from Vernonia anthelmintica willd (L.) seed counteracts stress-induced murine hair follicle growth inhibition

Version: 1 Date: 05 Aug 2019

Reviewer: Reviewer 2

Reviewer's report:

"PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

No - there are minor issues

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

No - there are major issues

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

No - there are major issues

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?

Maybe - with major revisions
PEER REVIEWER COMMENTS:

GENERAL COMMENTS: The study is very interesting, as the need for alternative agents promoting hair growth is very high. However, the article needs improvements in the writing style, but especially in some evaluations. I have the sense that the authors are not experts on hair follicle biology. Therefore, it would be important that the authors critically review the introduction and clarify which hair loss one could target with AVE, carefully read again the guidelines for the hair cycle analysis in mice, and include a proper hair cycle analysis.

REQUESTED REVISIONS:

1) Please, have the manuscript carefully checked by an native english speaker (see "so on" line 5 background, last line M & M "anagen Induction" subchapter)

2) Add in the abstract that AVE has been already shown to stimulate hair growth (this is a very important background info)

3) Explain the rationale why the study was performed in the abstract and introduction (why would AVE have an effect on the release of neuropeptides?)

4) Background: do not generalize hair loss and specify for which hair loss AVE would be promising (i.e. stress seems to be involved in alopecia areata, telogen effluvium, etc. but not in androgenetic alopecia)

5) The sentences regarding the central nervous system is not really relevant to make the link with the aim of the study. Please, revise adding information on stressed-mediated hair loss (explaining the role of SP and CGRP), how AVE promotes hair growth, and what other property of AVE suggests that it could counter-act stressed-induced hair loss.

6) Line 23, the sentence starting with "In C57BL/6", needs to be corrected as in all systems (human, mice, rat) melanogenesis is coupled with only anagen, and it is off during catagen and telogen.

7) Please, explain better why C57BL/6 is the optimal model, I do not buy what you have written, human is the ultimately good model. However, I would buy it if you write and quote that many study using this model focused on understanding the neuro-endocrine regulation of hair cycle, and that were used to test other compounds.
8) Line 12, page 5, you are generalizing the FDA approved drug, minoxidin and finasteride are not given to for all hair loss, please specificy what it is relevant in the context of your study.

9) I am not aware with any adverse effect of minoxidil. Please, find supporting references.

10) M&M: It is missing the number of mice used for the study.

11) This is a very major issue: you did not use the correct criteria, despite of the fact that you quoted the correct guide, for the hair cycle analysis. Morphology of dermal appilla and sebaceous gland has nothing to do with hair cycle. Instead, morphology of the hair bulb, how deep is the hair bulb, number of proliferating hair matrix keratinocyte below Aubers line, etc. are the important criterias. Given this important issue, hair cycle should be re-analize following the instruction of the guide.

12) Although the colour of the skin is a very good indicator of hair cycle stage, it cannot be use as the only criteria for the hair cycle analysis.

13) The term ""degenerative morphological changes"" does not exist. What do mean with this: catagen promotion or hair follicle cytotoxicity?

14) It is important to quantify the number of Ki-67+ hair matrix keratinocytes elow the Aubers line as further confirmation of the ahir cycle result.

15) K19 immunostaining is totally unspecific in the inner root sheath. I am not sure it is detecting K19+ cells in the bulge. Please, show higher magnification of bulge outer root sheath, and use other markers better suited for detecting bulge stem cells, i.e. K15 and CD34. Yet, it is important to describe why did you evaluate this marker because it is not really relevant for anagen prolongation but more for anagen initiation, i.e. telogen to anagen transition.

15) Also for SP and CGRP the images are not convincing. You are indicating as positive staining the unspecific staining on the arrector pili muscle. Show higher magnification, better quality picture. To be sure that what you are detecting are indeed nerve fibers, you should use also PGP9.5 as marker.

16) I am also not convinced about the down-regulation of NK-1R expression, please confirm this with immunohistology. " 
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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