Reviewer's report

Title: Hypericum perforatum treatment: effect on behaviour and neurogenesis in a chronic stress model in mice

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Reviewer: Oliver Grundmann

Reviewer's report:

The manuscript submitted by Crupi et al. entitled "Hypericum perforatum treatment: effect on behaviour and neurogenesis in a chronic stress model in mice" describes some important and interesting research findings that are worth being reported. However, there are some necessary corrections and clarifications that should be addressed by the authors before publication.

Discretionary revisions:

1. BrdU as a key word does not seem to be very specific - the authors may consider to eliminate this keyword.

2. The Latin name of St. John's wort should also be italicized in the abstract.

3. The last sentence of the background section should probably end with "correlations" instead of "implications" since behaviour is correlated to the morphological changes observed but not an implication of these changes.

4. Figure 4A may not be necessary - the application of BrdU 2 hours prior to sample collection can be mentioned in the methods section.

Minor Essential Revisions:

1. In the Conclusions section of the Abstract, the authors state that morphological adaptations occurred in both newborn and mature hippocampal neurons. In the paper and the methods section, only adult mice are mentioned that were actually analyzed. This should therefore be clarified.

2. In the background section, the authors do not mention flavonoids as potential active components in Hypericum extracts. This has been shown by Butterweck et al. in mice and rat models and should therefore be included (specifically hyperoside and isoquercitrin).

3. Under "Animals" the care guidelines should probably state NIH instead of NHI.

4. Usually, standardized Hypericum extracts contain a certain amount of flavonoids that are quantified by quercetin and its conjugates - this should be clarified in the manuscript. Kaempferol should be written correctly.

5. In the immunohistochemistry section, when the term DG is first mentioned, it should be written as dentate gyrus followed by the abbreviation (DG). Thereafter

it can be written as DG.

6. On figure 4H, the single star above the cort- vehicle group should be removed.

Major compulsory revisions:

1. Under "drugs and treatment schedule" the vehicle control should be defined - was it simply water? Did the animals who did not receive Hypericum still receive DMSO/saline injections?

2. The authors state later that each group actually had n=8 animals - but in the methods section, each group had n=16 animals. The assumption that the intial two groups, cort- and cort+ each have n=16 animals, are then each split up into Hyp+ and Hyp- to give the final n=8 animals/group should be clarified here.

3. The explanation of the 4-point scale for fur condition is insufficient - only 2 conditions have been mentioned (0 and 1) - what about the other 2?

4. How were the hippocampal samples retrieved? Which method was used and was the whole brain or only the hippocampal region fixed in formalin solution? Which magnification was used for detection?

5. The "Statistical analysis" section is not sufficient. There are overall four groups of animals and as such, a simple t-test is not suitable to compare between these groups. An ANOVA test needs to be included, either with a pair-wise Bonferroni adjustment for the number of pairwise comparisons, or an overall multiple comparison test such as Newman-Keuls, HSD, or Tukey's LSD.

6. Under results, the deterioration of the coat state was not shown in the figures - this should be mentioned or data included. Also, figure 1 only compares 3 groups - cort-, cort+, and cort+/Hyp+. Overall, there were 4 groups and the group cort-/Hyp+ is not included. The authors should give a justification why this group has not been included or change figure and correct the results and discussion section accordingly.

7. The DCX+ cells were not significantly different between cort+/vehicle and cort-/vehicle - this is not stated correctly in the paragraph "immunohistochemistry results".

8. Figure 2 - as mentioned before - should include the non-stressed Hyp+ group.

9. In general, figure legends should be descriptive, figure 3A does only offer interpretation without any description of what is displayed.

10. In figure 4I and 4L, the two photomicrographs shown should be identified as to which group they belong.

Overall, the experimental design is well prepared and conducted. The results are promising and help to further elucidate the effects of Hypericum extracts on stress and morphological adaptations in critical brain regions related to the HPA axis.

The authors should briefly discuss the metabolic difference between oral and intraperitoneal administration. Intraperitoneal administration does circumvent the intestinal metabolism but still subjects compounds to hepatic metabolism. The oral route of application would best reflect the metabolic processes involved in Hypericum extract administration as it is exclusively given orally to patients for the treatment of depressive disorders.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.