

## OPEN PEER REVIEW REPORT 2

**Name of journal:** Neural Regeneration Research

**Manuscript NO:** NRR-D-21-00908

**Title:** Icaritin Ameliorates Memory Deficits through Regulating Brain Insulin Signaling and Glucose Transporters in 3×Tg-AD Mice

**Reviewer's Name:** Marta Valenza

**Reviewer's country:** Italy

### COMMENTS TO AUTHORS

The authors study the beneficial effect of chronic oral administration of the flavonoid Icaritin in ameliorating behavioral and molecular signs of Alzheimer's disease pathology using a triple transgenic murine model of AD.

The results seem interesting, however, I am afraid that the statistical analysis carried out was not appropriate. I believe that a two-way ANOVA should have been used since 2 independent variables are present, genotype and treatment. As for now, I am not sure that the conclusions are supported by the data.

Another issue is the vehicle chosen of oral administration: double-distilled water should not be used for oral administration.

Some suggestions are included below to improve the manuscript:

- Numerous language issues detected throughout the manuscript with exception of the discussion.

Few examples: - in the graphical abstract: sentence in the blue rectangle.

- in the abstract: <<in an AD model of triple transgenic AD>> ,  
<<reversed neuronal nuclei>>

I suggest having a native language writer to edit the manuscript.

- Low quality figures. Increasing dpi could help. The fonts chosen are so small, increasing them would help the reader.

- Figure 1: change <<and>> with <<OR>> in the sentence explaining the treatment (e.g. vehicle OR ica)

- Figure 5, change the direction of graph C.

- Legend to figures: magnification mentioned for IF or IHC figures seems too big: 400x or 200x are mentioned, but figures clearly show figures taken at lower magnification, they are likely 4x  
The method sections should be greatly improved:

- Please add references for each method used

- I have some doubts about the sentence at line 30, below legend to fig1, saying that 3×Tg-AD mice exhibit Abeta and tau pathology as early as 3months. Please add reference for this. As far as I know signs of tau pathology have been documented at 6 months, later than Abeta deposits.

- please explain why only male were included in the experiments considering that AD is prevalent in females

- 2.1 SPF acronym not explained

- Please show data of body weight change for the 5 months of administration

- Please specify what kind of oral administration was used: gavage?

- 2.2 Please edit the first sentence <<Some mice>> specifying the number of animals. Similarly, in the last sentence <<The others>>



- Please specify dose of chloral hydrate in mg/kg
- <<the entire cerebral cortex was quickly separated at low temperature>>. Please clarify
- All methods miss information about what was analyzed and how:
  - Y maze: how did you calculate and analyzed spontaneous alternation?
  - HE, Nissl and IF staining: after visualizing the staining under the microscope, how did the authors analyze the experiment? Many details are missing, as the objectives and camera used. How many slices per animal were stained and analyzed? What area was stained?
  - WB: was the entire cortex homogenized? Concentration of each antibody? What analysis was performed after acquiring the image?
- The Results section misses to mention the result of each ANOVA.