# Review of: "Targeting Alzheimer's disease hallmarks with the Nrf2 activator Isoeugenol"

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Potential competing interests: No potential competing interests to declare.

Dear Editor,

I have meticulously reviewed the manuscript titled "*Pharmacological Characterization of Isoeugenol as a Potential Treatment for Alzheimer's Disease*" by Ana Silva et al. I commend the authors for their thorough investigation into the potential therapeutic benefits of Isoeugenol in Alzheimer's disease (AD). The study provides valuable insights into the activation of the Nrf2 pathway and its effects on AD hallmarks.

**General Comments:**

The manuscript presents a compelling demonstration of Isoeugenol's pharmacokinetic and pharmacodynamic profile, showcasing its ability to activate Nrf2 *in vitro* and mitigate AD-related pathologies with safety. The abstract is clear, with well-chosen keywords, and the introduction flows seamlessly. However, I recommend clarifying the chosen statistical analysis, especially when analyzing behavior. Additionally, I am curious about the effects on the activation of NRF2 and potentially the AKT pathway in mice.

**Abstract:**

The abstract effectively encapsulates the key findings of the study.

**Introduction:**

While the introduction appropriately highlights the importance of investigating novel therapeutic avenues for AD, a multifactorial disease, I suggest incorporating information on the role of tau and hyphosphorylation of tau, particularly in the early stages of AD, for a more comprehensive overview. In addition, a more in-depth discussion of the limitations of both long-established and recently FDA-approved therapies, along with the potential advantages of exploring alternative molecules possessing easy administration routes, minimal side effects, and a requirement for smaller quantities, as suggested by the Isoeugenol study, would enhance the introduction.

**Methods and Results:**

The study design, including in vitro and in vivo experiments, is well-structured, and the integration of behavioral and biological evidence strengthens the overall argument. However, the in vivo modulation of the NRF2 pathway should be investigated.

The number of mice used in the study might be considered limited. Please provide justification for the chosen sample size and discuss if a power analysis was conducted.

My main concerns lie with the choice of statistical tests across several experiments, particularly those for behavior analysis. Ensure uniformity in the choice and justification of statistical tests throughout the manuscript. Specifically, clarify the use of One-way ANOVA and t-tests in different figures, and provide information on the significance of ANOVA before proceeding with multiple comparisons. The authors used One-way ANOVA with either Tukey's or Dunnett's multiple comparisons (Fig 2 vs Fig 3, for example). The authors used t-test and One-way ANOVA in Fig 6, 8, 13, 16, 17, while only One-way ANOVA should be used. Authors should provide information on the significance of ANOVA before proceeding with multiple comparisons. Two-way ANOVA should be used for the analysis described in Fig 8 – G.

**Discussion:**

The discussion could benefit from more references justifying the results of the study to existing literature. For example, regarding the anxious phenotype observed by the authors in young Tg veh mice compared to wt, "since anxiety is a prodromic symptom of AD."

The effects of Isoeugenol on AB42 and AB40 levels, which differ between plasma and the brain, deserve further discussion. Authors exhibited a positive impact on cognitive symptoms in mice, while reduction is observed only in plasma.

**Conclusion:**

In conclusion, the manuscript presents a compelling case for Isoeugenol as a potential therapeutic agent for AD. Addressing the mentioned concerns and refining certain aspects will undoubtedly strengthen the overall impact of the study.

I recommend acceptance with revisions, emphasizing the need for clarity in statistical methodologies, additional references in the discussion, and a more streamlined presentation of results.

Sincerely,

Benoit Souchet, PhD