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.

The paper of Silva et al is an experimental research on the possible benefice of isoeugenol as a therapeutic agent against AD. Well written, the subject is clearly introduced followed by a good presentation of the objectives of the study.

Two (**minor**) remarks, maybe obsolete later on (?): (i) isoeugenol is also known to have a cancerous side (https://doi.org/10.1016/j.yrtph.2022.105280). This dark side should be at least cited and the readers would be warned not to fall from Charybdis (AD) to Scylla (cancer). I also believe it is somewhat linked to aspartame; (ii) Again, this remark may be obsolete later in the paper, but it is worth mentioning it (as zillions of people actually taking it as a sweetener, isn't it?) (International Agency for Research on Cancer. Volume 134: Aspartame, methyleugenol, and isoeugenol. Lyon, France; June 6–13, 2023. IARC Monogr Identif Carcinog Hazards Hum).

I believe isoeugenol is a natural compound, from plant source. Please state this point clearly in the introduction.

The mechanism of regulation of nrf2 – briefly and clearly summarized – reminds me of the similar behavior of another set of proteins (thioredoxin (TRX)-interacting protein). It may be worth (i) citing it as a similar system that also attracted – unsuccessfully? – the Pharma industry and (ii) knowing that such systems were explored elsewhere and tricks used there might be interesting to import in this domain.

Fumarate was also 'discovered' by Talalay's group as the active moiety of his obsession with sprout-based therapeutical benefit (Spencer SR, Wilczak CA, Talalay P. Cancer Res. 1990 Dec 15;50(24):7871-5. PMID: 2123743).

I found the concentration of Iso used in the study a bit concerning, as ¼ mM seems a stratospheric concentration for any compound. Please elaborate on that.

Finally, as a general comment I found the discussion a bit long – it looks like the translation of a thesis discussion. I am afraid, despite the many information buried there, that it will escape the reader's attention. My suggestion would be to cut it by half, maintaining the key points clearly state. I did not see a discussion on the concentration used (very high) and the risk thereof, nor did I find a reference on the sweetener composition – I believe iso is part of it. Please, elaborate shortly.

Minor At the end of the 4th paragraph of the introduction, I suggest to state new hypothesis such as Gould et al's one in JCI (doi: https://doi.org/10.1172/JCI162120.)

Minor: (8th paragraph, intro) The term "in vitro" is confusing because it can design a-cellular systems as well as cellular systems. I'd suggest to use "in cellulo" rather than in vitro, when relevant – i.e. in cellular systems.

Minor: part 3.1 “in chimico”???

Major: results 3.1 the use of sub-millimolar concentrations of any chemicals is a dangerous path. Please elaborate on this.

Major: I’d suggest a similar compound to be used in cellular model(s) such as eugenol at these concentrations, to be sure that the effect is not a concentration-only effect.

Major: (Figures 3, 4, 5) I have a bad time with blots presented as stamp collections. Please, provide at least as suppL. Material the whole blot. This permits the reader to judge of the quality of the Ab, in the experimenters’ hands..
(Incidentally, some journals refuse such presentations)

Minor: (Figures 6 & 7) please state how one can go from 250/500 µM to 50 mg/kg?? This is mentioned at the bottom of page 23, but required to be presented and discussed earlier in the paper.