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 article presents a study that employs isoeugenol, a Nrf2 activator, to investigate possible therapies for Alzheimer's disease. The ability of the body to maintain equilibrium in processes linked to oxidative stress, energy metabolism, inflammation, and protein regulation is known to be disrupted by Alzheimer's disease. The study investigates the use of the electrophilic chemical isoeugenol to activate Nrf2 and reverse some of the conventional symptoms and signs of Alzheimer's disease. According to the study, isoeugenol activates Nrf2, has good pharmacokinetic and pharmacodynamic attributes, and possesses anti-inflammatory and antioxidant abilities. Isoeugenol has shown promise in lowering levels of Aβ peptides, which are linked to Alzheimer's disease, in both laboratory cell cultures and animal models. Furthermore, it ameliorated memory impairments in aged mice, indicating possible advantages even in the later phases of the disease. The safety of isoeugenol was also assessed in the study, and it was discovered that it had no negative impacts on metabolic parameters or significantly altered the histology of important organs. The study concluded by showing that isoeugenol may have therapeutic benefits for Alzheimer's disease in mice. It demonstrated anti-inflammatory properties, decreased Aβ peptide levels, and enhanced cognitive performance. The manuscript provides an impactful research work and should be considered for publication after incorporating the comments given below:

1. Introduction part is too lengthy, it can be reduced to 1000-1200 words. First 2-3 paragraphs are not very specific to the title of the paper.
2. **In Fig. 2. Effect of Isoeugenol on Aβ peptides levels in neuronal cells.**Why there is no any effect of Iso on N2a-APPswe group? Give the reason.
3. **Fig. 3. Effect of Isoeugenol on AKT, GSK3β and Nrf2 activation in AD neuronal cells.**Please give the endogenous for all the western blots in this section. It seems that in fig. 3(B), images of blot do not depict the graph, there is different expression in graphs and images. pAKT/AKT levels are seems to be nearly same in all the groups in blot images. Fig. 3 (C), N2a-wt and N2a-APPswe blots images also depicting the same expression of pGSK3β/ GSK3β in all the groups. Show the raw data for these blots and their analysis data. In fig. 3 (D), blots are again different from their graph. Please provide the raw file for all and analysis file. In fig. (E), Please provide the raw data. I think it should be cytoplasmatic instead of citoplasmatic in graph figure.
4. **Fig. 4. Effect of Isoeugenol on Nrf2-dependent antioxidant Hmox1 gene expression and HMOX1 protein levels in AD neuronal cells.** In fig. 4 (C), HMOX1 blot having no bands or bands are not visible. Why there is no change in RNA level (Fig. 4 A and B) as there is a change in protein expression in HMOX1? Explain?
5. **Fig. 5. Effect of Isoeugenol on inflammatory parameters in microglia cells exposed to LPS.**Fig. 5 (E),

endogenous and pro-IL-1β blots seems to be from different blots. Give endogenous from the same blot.

6. **Fig. 6. Effect of Isoeugenol on demographic and metabolic parameters of 6 mo AD mice.** Exposure of Iso causing change in body weight of mice. Explain?
7. **Fig. 8. Effect of Isoeugenol on locomotor activity and cognitive function of 6 mo AD mice.** The fonts of images are not visible. Why Iso is not causing any improvement in locomotor activity?
8. **Fig. 10. Iso effect on 11 mo WT female's metabolic and biochemical parameters.** There is a difference in LDL-C in Isi and Control group (E), give proper reason.
9. **Fig. 13. Iso-induced gene expression alterations in the cortex and hippocampus of WT and AD mice.** The level of Cortex HMOX1 (C) is different from that in Fig.4.
10. What is novelty of the given research as already many publications are there showing Iso therapeutic potential in neurodegenerative diseases?