OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-20-00698
Title: PSGF Protects Cultured Hippocampal Neurons against Glutamate-induced Cytotoxicity via regulating NMDA receptors
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COMMENTS TO AUTHORS

This manuscript investigates the neuroprotective effects of Polygalasaponin F (PGSF), a triterpenoid saponin monomer isolated from Polygala japonica, a traditional Chinese medical herb. To this end, the authors tested the effects of different concentrations of PGSF on hippocampal cells exposed to excitotoxic levels of glutamate. Thus, PGSF prevents glutamate-induced cell death, calcium overload, NMDA-mediated EPSC, regulation of NMDAR subunits expression and suppression of pro-survival signals. The entire work is very interesting, although some revisions should be made to improve the impact of the manuscript.

- Title: please, substitute the acronym PSGF with the name of the compound (i.e., "Polygalasaponin F protects cultured….")

- Page 2, lines 3-5: please, rephrase the first sentence of the abstract taking into account that NMDA receptor activation by excitatory aminoacids produces different forms of cell death, including apoptosis and necrosis.

- Page 2, line 22: being an in vitro study, I would suggest to replace "dose-dependent" with "concentration-dependent" throughout the manuscript (e.g., see also page 8, lines 11, 16 and 41, etc.)

- In figure 1D the authors represent a concentration-response curve, though their approach is inaccurate since these graphs should be semi-logaritmic (i.e., linear for y-axis, log for x-axis). Moreover, as it is, this graph reproduces the same data of figure 1C.

Statistical analysis (page 7): the authors should specify (here and in figure legends) how many replicates were used to obtain mean values. Moreover, the statistical test used is inappropriate for multiple comparisons: ANOVA followed by Dunnett's or Tukey's should be used instead.

Reference [18] (Sun et al 2012) demonstrates that PSGF is able to activate NMDAR. The discrepancy between the work of Sun and the major findings of this manuscript should be adequately discussed.

A very recent work demonstrates the neuroprotective effects of PSGF (Xie et al. Polygalasaponin F inhibits neuronal apoptosis induced by oxygen-glucose deprivation and reoxygenation through the PI3K/Akt pathway Basic Clin Pharmacol Toxicol 2020 Sep;127(3):196-204. doi: 10.1111/bcpt.13408). I strongly suggest to include this reference and to opportunely discuss it in the text.

Page 12, line 42: the authors state that "PGSF is a low-affinity NMDAR antagonist", based on the evidence that it "partially" suppresses NMDAR-mediated EPSP. This statement should be rephrased, since the observed effect may actually derive from the submaximal concentration of PGSF used. In fact, to demonstrate "low-affinity" the authors should use receptor binding techniques, otherwise they cannot keep this statement as it is.