Author's response to reviews

Title: In vitro neuroprotective potential of four medicinal plants against rotenone-induced toxicity in SH-SY5Y neuroblastoma cells

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The authors would like to thank all the reviewers for taking the time to review our submission. We appreciate all their valuable input and tried to accommodate all of their opinions and suggestions in the revised manuscript.

Reviewer 1:

- **Comment 1**: “Untreated cells”, referred to in the manuscript actually referred to vehicle treated controls. Amendments were made to reflect this in the manuscript.

- **Comment 2**: All cell viability experiments were performed after 72 h exposure. No cell viability experiments were conducted after only 24 h of exposure. The relevant section has been modified to try and clarify this issue.

  Subsequent mechanistic experiments were carried out at a concentration of 50 nM of rotenone because rotenone induced approximately 50% cell death at this concentration. It is necessary to induce some degree of cell death in order to evaluate changes brought about by rotenone and how the plant extracts may counter these. If there is too little cell death then subtle changes in cellular function may not be detected in mechanistic experiments. On the other hand, if too much cell death is observed then there will not be any cells left to use to evaluate the changes brought on by rotenone exposure. For this reason the authors selected the rotenone concentration that produced approximately 50% cell death.

  The authors further chose to use an exposure time of only 24 h for mechanistic studies and not the 72 h at which cytotoxicity was evaluated. Cytotoxicity endpoint assays only assess the final extreme of a series of pathological events that eventually lead to cell death. Trying to evaluate these early pathological events at the point of cell death would be of no value since these pathological events may have already ended. For this reason the authors decided to use a pre-cell death time point (24 h) to evaluate mechanistic changes in cellular functioning.

- **Comment 3**: The reason for the difference between the calculated LC\(_{50}\) and the observed LC\(_{50}\) of rotenone is a matter of experimental and mathematical error. The calculated LC\(_{50}\) was obtained from fitting a mathematical model to the observed data. Although this is currently the best, and only, way of calculating this parameter, it is not without fault and the resulting value remains only an estimation of reality. It is not possible to completely reproduce reality using a mathematical model as there are too many variables that come into play. This is further confounded by experimental error, which we can minimize, but not eliminate. For this reason there will always exist some discrepancy between estimated and observed values. Being well aware of this fact, the authors tested the calculated LC\(_{50}\) value to confirm its accuracy and it was found not to be true. Rather, a concentration of 50 nM of rotenone was observed to produce the desired result and was therefore used in all subsequent experiments. As for the plant extract concentrations used in the study, the prerequisite was for them to be non-toxic. The final concentrations used were below the calculated LC\(_{50}\) values and, more importantly, were also observed in the initial dose-finding experiments to actually be non-toxic.

- **Comment 4**: The reason for performing the mechanistic assays at a different time point than cytotoxicity endpoint assays was already discussed under Comment 2 in this rebuttal.

- **Comment 5**: Corrections were made to the relevant section.

- **Comment 6**: The manuscript already describes how the LC\(_{50}\) values for the different plant extracts were obtained, exposure to data reduction and analysis. As the manuscript already contains a lot of figures (five in total) the authors did not include the dose-response curves of the individual plant extracts because this
will not add any information that is not already in the manuscript. If the journal wishes to add these two figures to the manuscript, the authors will do so.

- **Comment 7:** The suggested amendment was made.
- **Comment 8:** Corrections were made to all figure legends.
- **Comment 9:** This section was modified to try and clarify the relevant methods.

**Reviewer 2:**

- **Comment 1:** The discrepancy regarding the corresponding author has been corrected.
- **Comment 2:** A paragraph has been included in the beginning of the Results and Discussion section which explains the reason for selection of the in vitro assays, with relevance to neuroprotection.