

Response to Reviewer 1 Comments

We would like to express our sincere thanks to the reviewer for the valuable comments. We corrected the manuscript according to the suggestions as follows.

Point 1: Abstract should be worked on to give the reader a better sense of all that is being examined eg testing the different AXT and formulations.

Response 1: More details were added in the abstract as shown in the red highlight.

Point 2: English needs a little work through the paper. Eg. In the abstract it says “biochemical histological analysis” this may mean Biochemical and histological

Response 2: We have already checked some grammatical errors and some typing mistakes. In the revised manuscript all corrections were made as well as editing throughout the manuscript by native speaker. In addition “biochemical histological analysis” was changed to “biochemical and histological analysis”

Point 3: figure one would be better if labels on the x axis instead of just the legend. It is hard to read the legends esp if the bar is small. This is true for other figures too, eg labels on x axis for figure 7 graphs

Response 3: We did correct the label on x axis for all figures.

Point 4: Morris water maze data: states that “Time spent in the target quadrant (target quadrant occupancy) and number of times that the animal crossed the position where the platform was placed (crossing times) were recorded.” But this is not clear from Figure 1 which shows escape latency and retention times. It is unclear what they mean by retention time? and escape latency is time to platform but they are doing a probe trial with no platform.

Response 4: Morris water maze test in 4.5.1 was rewritten to make it more comprehensible.

Point 5: What is swimming trial? Is this with a viable platform? (Yes)

Response 5: We apologize for an unclear writing about the method part. Let us explain a bit more herein. The concept behind this test is that the animal must learn to use distal cues to navigate a direct path to the hidden platform when started from different, random locations around the perimeter of the tank. Swimming trial or probe trial means start positions using distal locations for which the goal (platform) is located. In our experiment, we used four start positions, each was in quadrants except the one containing the platform.

References.

1. Chonpathompikunlert P, Wattanathorn J, Muchimapura. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal

model of cognitive deficit like condition of Alzheimer's disease. Food and Chemical Toxicology 48 (2010) 798–802.

2. Markowska AL, Long JM, Johnson CT, Olton DS. Variable-interval probe test as a tool for repeated measurements of spatial memory in the water maze. Behav Neurosci. 1993;107: 627–632.

3. Sutherland RJ, Chew GL, Baker JC, Lingard RC. Some limitations on the use of distal cues in place navigation by rats. Psychobiology. 1987;15:48–57.

4. Charles VV and Michael TW. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006; 1(2): 848-858. doi: [10.1038/nprot.2006.116](https://doi.org/10.1038/nprot.2006.116)

Point 6: water maze task is 5 days long just for the testing, but state tested on day 29 and euthanized on day 31. How is that possible?

Response 6: As indicated in Point 4, morris water maze test in 4.5.1 was rewritten to make it more comprehensible.

Point 7: Were the same animals used in all tasks? Were the same animals tested on day 14 and 29? Repeating learning and memory tasks is not a good practice.

Response 7: Thank you very much for your comment. We used the same animal in all groups for checking the effect of natural compound passing through therapeutic dose after oral administration via behavior changing or not which depends on time by time because it is not an invasive method. We have the control animal for comparison any time point to make the data more reliable. Moreover, several publications using behaviors checking for several time point are in common as the following references.

1. Wattanathorn J, Chonpathompikunlert P, Muchimapura. Piperine, Priprem A, Tankamnerdthai O. Piperine, the potential functional food for mood and cognitive disorders. Food and Chemical Toxicology 46 (2008) 3106–3110.

2. Boonruamkaew P, Chonpathompikunlert P, Vong LB, Sakaue S, Tomidokoro Y, Ishii K, Tamaoka A, Nagasaki Y. Chronic treatment with a smart antioxidative nanoparticle for inhibition of amyloid plaque propagation in Tg2576 mouse model of Alzheimer's disease. Scientific Reports. 2017, 7 Article number 3785. DOI: [10.1038/s41598-017-03411-7](https://doi.org/10.1038/s41598-017-03411-7).

3. Hyeon YL, Jin BW, Youn SJ, Nam YK, Myong KK, Choong JM. Cognitive-enhancing effect of Aronia melanocarpa extract against memory impairment induced by scopolamine in mice. Evidence-Based Complementary and Alternative Medicine. Volume 2016, Article ID 6145926, 7 pages

Point 8: figure 8 is not measuring plaques but immune positive A-beta as percentage area. Label needs changing

Response 8: We changed the label of Y-axis of figure 8B, C, and D from “A β plaque (% area)” to “positive staining of β -amyloid (% area)”.

Point 9: Orally administered? Does this mean Gavage or in Diet?

Response 9: Oral administration is a route of administration where a substance is taken through the mouth. Per os (P.O.) is sometimes used as an abbreviation for medication to be taken orally. So, orally administered means oral gavage.

Point 10: Unclear about brain preparation. 4.7.1 and 4.6. It is very unclear how many animals are used here. There are two different methods of brain extraction but no mention of different cohorts.

Response 10: More details were added to make it clearer on numbers of animal used as indicated in 4.4, 4.6 and 4.7.1.

Point 11: What stereological software was used for cell counts?

Response 11: Image J (Java 1.4, USA.) was used as stated in 4.7.1 and 4.7.2.