Peer Review File

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Comment 1: Section 3.2.2, 3rd paragraph: The authors say that the MWM is performed before and after treatment with icariin. That is not typically how these experiments are done especially as MWM outcomes can be influenced by prior experience. Typically, there are untreated and treated groups of animals that are compared at the same time. The authors should check the different studies to determine which approach was used.

Reply 1: Thank you for your valuable comments. As per your suggestions, we checked some studies and what we want to express is comparing and analyzing some changes in AD model animals treated or untreated with drugs. We have modified our text as advised (see Page 9, line 200).

Changes in the text: By comparing and analyzing the changes in spatial cognition, learning and memory of AD model animals treated or untreated with drugs, we can evaluate the effectiveness of the intervention, in this case, icariin, to alleviate AD.

Comment 2: Section 3.2.3, 2nd paragraph, 3rd line: define IR

Reply 2: Thank you for notifying us. We have modified our text as advised (see Page 10, line 229).

Changes in the text: Aβ immune-reactivity areas.

Comment 3: Section 3.2.3, pg 12, line 11: Did TGB-b go up or down? This is not clear.

Reply 3: Thank you for your valuable comments. Studies have shown that TGF-β signaling elicits complicated effects (e.g., beneficial or detrimental) in Alzheimer's disease [1-2]. We checked the cited article [3] and its results showed that the TGF-β1 level in brains of transgenic mice was increased. We have modified our text. (see Page 12, line 268-271).

Changes in the text: Zhang Z. et al. reported that icariin inhibited the activation of microglial ($P< 0.05$) and the level of TGF-β1 ($P< 0.05$) in the hippocampus and cortex, and alleviated behavioral deficits.

[1] Das P, Golde T. Dysfunction of TGF-beta signaling in Alzheimer's disease. J Clin Invest 2006;116:2855-7.

[2] Lesne S, Docagne F, Gabriel C, et al. Transforming growth factor-beta 1 potentiates amyloid-beta generation in astrocytes and in transgenic mice. J Biol Chem 2003;278:18408-18.

[3] Zhang ZY, Li C, Zug C, et al. Icariin ameliorates neuropathological changes, TGF-beta1 accumulation and behavioral deficits in a mouse model of cerebral amyloidosis. PLoS One 2014;9:e104616.

Comment 4: Section 3.2.3, pg. 12, line 15-16: Explain relevance of PPAR-g to microglial activation.

Reply 4: Thank you for your suggestion. PPAR-γ is an important nuclear receptor, which is highly expressed in microglia[3]. Studies confirmed that PPAR-γ can attenuate M1 activation of microglia and mediate its transformation to the M2 phenotype[4,5]. In the article we cited, icariin attenuated the levels of M1 phenotype markers and increased the level of M2 phenotype markers by activating PPAR-γ. We have added references and modified our text (see Page 12, line 271-276).

Changes in the text: Studies confirmed that PPAR-γ can attenuate M1 activation of microglia and mediate its transformation to the M2 phenotype(52,53). Wang Y. et al.(15) found that treatment with icariin decreased the levels of IL-1β, TNF-α and IL-6, and increased the levels of IL-4, IL-10 and TGF-β in the hippocampus and prefrontal cortex of APP/PS1 mice ($P< 0.05$). These changes may be related to PPAR-γ inhibiting the activation of M1 microglia.

[3] Malchiodi-Albedi F, Matteucci A, Bernardo A, et al. PPAR-gamma, Microglial Cells, and Ocular Inflammation: New Venues for Potential Therapeutic Approaches. PPAR Res 2008;2008:295784.

[4] Saijo K, Crotti A, Glass CK. Regulation of microglia activation and deactivation by nuclear receptors. Glia 2013;61:104-11.

[5] Li X, Guo Q, Ye Z, et al. PPAR gamma Prevents Neuropathic Pain by Down-Regulating CX3CR1 and Attenuating M1 Activation of Microglia in the Spinal Cord of Rats Using a Sciatic Chronic Constriction Injury Model. Front Neurosci 2021;15:620525.

Comment 5: Section 3.2.3, pg. 12, 2nd paragraph, line 6: This should be "oxidation" not "anti-oxidation".

Reply 5: Thank you for your correction. We have modified our text as advised (see Page 12, line 282-284).

Changes in the text: ……, are the first line of defense against oxidation in the body.

Comment 6: Section 3.2.3, pg. 13, 1st paragraph: The studies described in this paragraph appear contradictory. One says that icariin increases the activity of SOD and the others say that icariin decreases SOD expression. Please clarify.

Reply 6: Thanks for your valuable comments. We rechecked the article and confirmed that icariin increased SOD-2 mRNA expression in AD models. We have modified our text as advised (see Page 13, line 290-291).

Changes in the text: Treatment with icariin inhibited oxidative stress by increasing SOD-2 mRNA expression ($P< 0.05$).

Comment 7: Section 3.2.3, pg. 13, 2nd paragraph, line 1: The studies do not show that icariin alleviated cognitive impairment by regulating autophagy. They only show that there is a correlation between the two. Please rephrase.

Reply 7: Thank you for your comment. We have modified our text as advised (see Page 13, line 293-294).

Changes in the text: Two studies indicated that the alleviation of icariin on cognitive impairment is related to autophagy.

Comment 8: Discussion, pg. 15, 2nd paragraph, lines 8-9: The SAMP8 mice are not particularly short lived. They live to be at least 13 months old which is plenty of time for the testing of protective compounds. Furthermore, in contrast to the transgenic AD

mice, the SAMP8 mice can be considered a model of sporadic AD. Since over 95% of human cases are sporadic, it could be argued that the SAMP8 mice are a much more relevant pre-clinical model. The authors need to mention these points.

Reply 8: Thank you for your valuable comments. We have modified our text as advised (see Page 15, line 348-351).

Changes in the text: SAMP8 mice are considered as a model of sporadic AD. Since over 95% of human AD patients are sporadic, it could be argued that these mice are a much more relevant pre-clinical model. But it is relatively expensive.