Author’s response to reviews

Title: Stimulatory effect of icariin on the proliferation of neural stem cells from rat hippocampus

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Author’s response to reviews:

BCAM-D-17-00854

Stimulatory effect of icariin on the proliferation of neural stem cells from rat hippocampus

Xiaolong Fu; Shaoyu Zhou; Jingshan Shi

BMC Complementary and Alternative Medicine

Dear Mr. Fu,

Your manuscript "Stimulatory effect of icariin on the proliferation of neural stem cells from rat hippocampus" (BCAM-D-17-00854) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Complementary and Alternative Medicine.

Their reports, together with any other comments, are below. Please also take a moment to check our website at http://bcam.edmgr.com/ for any additional comments that were saved as attachments. Please note that as BMC Complementary and Alternative Medicine has a policy of open peer review, you will be able to see the names of the reviewers.

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amendments have been made to the manuscript text and where these can be viewed (e.g. Methods section, line 12, page 5). Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes. If you disagree with any comments raised, please provide a detailed rebuttal to help explain and justify your decision.

Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Instructions for Authors on the journal homepage.

A decision will be made once we have received your revised manuscript, which we expect by 09 Dec 2017.

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I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

Insop Shim, Ph.D

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Reviewer reports:

Jungwon Seo (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

In this study, the authors showed that icariin promotes the proliferation of neural stem cells from rat hippocampus. However, the study is preliminary and there are some critical concerns.

1. Experimental methods are not clear. How did you count the cells of neurospheres in Fig.3?

2. In addition, how did you count BrdU positive cells in Fig.4? Did you measure the fluorescence or directly count the cell number? Because neurospheres are not attached and are collection of the cells, the results will be changed by the cell count method. Please provide detailed method.

3. p21 is an inhibitor of CDKs and associated with cell cycle arrest. The roles of cyclin D1 and p21 are opposite regarding cell cycle regulation. There is no clear discussion about icariin-induced increase in p21 expression. Please discuss about the possible mechanism or function of p21 expression on NSC proliferation.

4. The mechanism of icariin-induced NSC proliferation should be more examined. Upstream molecules of cyclin D1 or p21 are well-known. Thus, authors can figure out the activity of upstream molecules i.e. Akt phosphorylation, CREB phosphorylation and so on.

5. If icariin promotes NSC proliferation, theoretically it is likely to inhibit NSC differentiation. Did authors research about differentiation effect of icaruin?

6. Please check English spelling. For example, multipotency in page 9.

Jariya Umka Welbat (Reviewer 2): BCAM-D-17-00854

Title: Stimulatory effect of icariin on the proliferation of neural stem cells from rat hippocampus

Minor comments
1. On page 7 line 54 "quantifing" should change to "quantifying".

2. On page 8 line 7, for this method "The effect of ICA on gene and protein expression of cyclin D1 and p21" should be separated as the gene expression was used to determine both cyclin D1 and p21 but protein expression was used to study only p21.

3. On page 10 line 58-60, "expression of mRNA and protein cyclin D1 and p21 were detected by quantitative RT-PCR and Western blot, respectively" should change as the gene expression was used to determine both cyclin D1 and p21 but protein expression was used to study only p21.

4. On page 12 line 23, "therefore investigated the gene and protein expression of cyclin D1 and p21" should change as the gene expression was used to determine both cyclin D1 and p21 but protein expression was used to study only p21.

5. The quality of images of Figure 2B and 4A are poor.

The authors' response letter BCAM-D-17-00854 R1 has been included as a supplementary file.