Author’s response to reviews

Title: Antitumor effects of Quercetin on Y79 retinoblastoma cells via JNK and p38 MAPK pathways

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Author’s response to reviews:

Nitin Telang, Ph.D (Reviewer 1): Overall, the manuscript is reasonably well written and contains good data on anti-proliferative and pro-apoptotic effects of Quercetin on Y79 retinoblastoma cell line. The study outcome provides convincing evidence for the involvement of the JNK and p38MAPK pathways in growth inhibitory efficacy. However, the following points need to be considered and appropriately addressed.

1. All the experiments have utilized an acute (24 hrs.) treatment of Quercetin. The extent of growth inhibition in response to long-term treatment is essential to justify potential clinical translation of the study outcome.

Response: We respect the suggestion provided by respected reviewer. The present work that we have submitted is a part of research work assigned for screening some identified compounds for their potential against RB. We have screened one of them and looking into the results we will extend our study ahead specifically for long term treatment of the same. The results of 24h exposure that we have obtained were encouraging and we will further test for long term exposure also. Presently we have presented whatever we have got from our experiments and we were guided for. I believe that the respected reviewer is convinced with the outcomes suggesting involvement of the JNK and p38MAPK pathways in growth inhibitory efficacy.

2. The CDK inhibitors p21 and p27 are also associated with the tumor suppressive function of p53. What is the status of p53 in the present cell culture model?

Response: We had not evaluated the status of p53, as we found reports supporting role of p21 and p27 in providing sufficient evidence in proving the said activity we opted for p21 and p27.
However we thank respected reviewer for the suggestion which is educative to us, we can include the same in our studies ahead.

3. The tumor suppressive effects of the RB gene are known to be effective via cyclinD-CDK4/6-pRB- E2F cascade. What is the status of this pathway in the model, and what is the response of Quercetin to this pathway?

Response: We had gone through some literatures such as (J Bone Miner Metab. 2008;26(6):551-60) which supported anticancer role of selected molecule QCT through MAPK-dependent mechanism hence we directed our experiments on the results of previous studies.

4. The rationale to examine cyclin D3-JNK-p38MAPK pathway needs to be stated, and its relationship with the traditional RB signaling needs to be discussed.

Response: We have included a paragraph in discussion section, if the respected reviewer finds it insufficient I request him to kindly specify the needed matter more clearly.

Sachiko Sugimoto (Reviewer 2): The referee would not agree to publish of this manuscript in BMC Complementary and Alternative Medicine as an original paper because some of reasons, there are….

This manuscript doesn't find out novelty. Because this manuscript is similar to a reference which title is Curcumin exerts antitumor effects in retinoblastoma cells by regulating the JNK and p38 MAPK pathways.

Response: Here we have identified the possible mechanism by which QCT exerts its anticancer effect on RB cells. There are number of studies identifying the mechanism similar to the present one, we believe that there can be similar mechanisms, existence of the said paper by respected reviewer explores the anticancer effect of Curcumin and not Quercetin.

1. Please raise the resolution all figure. Please make the title of axis more visible. In Figure 2A, is comment of X-axis correct? The referee think it is not incubation time but Quercetin.
Response: We have increased the resolution to 300dpi. The X-axis has been labeled incorrectly it is Quercetin and not incubation time, we have now made the correction.

2. As Figure 2B, the author should add the results of 0 μM of quercetin. If not, the author cannot analyze as correct data. In figure 2B and 2C, 4A and 4B, 5A and 5B, why are they displayed different concentration of quercetin each other? (ex. 0, 50, 100 or 25, 75, 100.)

Response: We regret for the typo error in displaying the concentration. It is now corrected; we thank the reviewer for identifying the same.

3. As Figure 6A and 6B, are they correct to show the results of Western blotting graphically? The referee think it is not match between image of Western blot and graph, especially Figure 6B. (As for, Quercetin ++)

Response: We found the interpretation done for creating graphs improper for the mentioned western blot. We have now corrected it, the graph represents the correct values now.

4. L.118, RAPMI : please correct RPMI

Response: The line has been modified for said correction.

5. L.138, 138, g/mL : Is it correct? or μg/mL??

Response: The typo error has been rectified.