Author's response to reviews

Title: "Citrus aurantium flavonoid" inhibits adipogenesis through the Akt signaling pathway in 3T3-L1 cells

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Author's response to reviews:

Dear Editor
BMC complementary and alternative medicine
Ms. No.: 8528225786519946
Title: “Citrus aurantium flavonoid” inhibits adipogenesis through the Akt signaling pathway in 3T3-L1 cells

Dear Editor,

Thank you for sending us the reviewer’s comments and your kind letter encouraging us to submit our revision. The reviewer’s are very supportive and had several minor comments to improve our manuscript. We have read and carefully considered the comments of the reviewer, accordingly we have revised our manuscript. We would like to thank the reviewers for their efforts and time, particularly, for useful comments to make our paper better. We believe that our manuscript has been substantially improved by this revision.

Thank you very much for your time and consideration.

Your sincerely,

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Reviewer #1

Major concerns:

1. All data were based on the hypothesis that major constituent of CAL was flavonoids. On page 9, lines 6-8 (from the bottom), the authors mentioned that CAL consists of.....However, in this study, there is no evidence that major component of CAL is flavonoids. This reviewer failed to find any descriptions for this fact from the current manuscript. The authors should clarify this issue.

   Answer: We would like to thank the reviewer's comments in preparation of the revised manuscript. The CAF extracts were supplied by Dr. Shin and Kim’s Labs and it was cited in this manuscript reference (21, 22). Kim’s paper has been published in last Month. Analysis of the individual components of CAL extracts by HPLC were conducted at Dr. Shin and Kim’s Labs, and CAL consists of many flavonoids, naringin, hesperidin, poncirin, isosiennsetin, hexamethoxyflavone, sineesytin, hexamethoxyflavone, tetramrthnl-o-isoscutellaein, nobiletin, heptamethoxyflavone, 3-hydoxynobiletin, tangeretin, hydroxypentamethoxyflavone, and hexamethoxyflavone.

2. Anti-adipogenic activity of CAF was clearly confirmed through essential molecular works. However, lipolytic activity of CAF was not fully verified. For example, LPL activity was not increased (Fig. 2B) even though released glycerol was increased (Fig. 4B) upon CAF treatments.

   Answer: Yes, we agree with the reviewer’s point. Lipolysis is the degradation of Triglyceride inside differentiated adipocytes. Lipolysis and lipogenesis are influenced by activation of LPL and HSL in adipocytes. Free glycerol release was measured to get an estimate of lipolysis, which was significantly higher in CAF treated adipocytes (Figure 4B), but the expression of LPL was not changed in the cell treated with CAF (Fig. 2). Other studies also showed that n-3 PUFA-rich perilla oil did not change LPL expression in rat adipose tissue although a reduced adipogenesis was observed (Okuno et al., 1997). Similarly, hydroxytorsiol from olive oil inhibits lipid accumulation during adipocyte differentiation, but not changes the expression of LPL (Warnke et al., 2011). We speculated that CAF play an important role in lipolytic regulation, however further studies are needed to determine its mechanisms.

3. Many typo and grammatical errors are found throughout the manuscript. Moreover, structure of the manuscript should be extensively modified to meet scientific standard. In DISCUSSION section, some redundant statements that
were already described in RESULTS section should be deleted. DISCUSSION section was not written in a continuous flow. For example, many expressions are not suitable for this section but suitable for INTRODUCTION section, and thus moved to INTRODUCTION section or removed (e.g. page 9, Citrus fruits are ……).

Answer: We would like to thank the reviewer’s comments. We have revised according the suggestions of the reviewer.

Minor points (the authors should have indicated line number!).
1. (Title) inhibits>inhibit, “Citrus aurantium” should be italized throughout the text.
Answer: The text is revised to clarify this issue.
2. Page 2: type-2>type 2
Answer: Text is revised as directed.
3. Page 4: sigma>Sigma, culture>cultured, changes>changed, Fetal Bovine Serum>fetal bovine serum, obtained>purchased, as described>as described previously. Please delete “The analyses of …Prof. Sung Chul Shin”
Answer: We would like to thank the reviewer’s comments. We have revised according the suggestions of the reviewer. Text is revised as directed.
4. Page 5: All descriptions for suppliers should be notes as: supplier’s name, city, (state), country. From their second appearance, they can be used with only supplier’s name without address. Intorgen>Introgen. Indicate addresses for Cell Signaling, Chemicon, and Jackson, etc.
Answer: Text is revised as directed.
5. Page 6, figure legends, and throughout the whole text: all “p” in statistical analysis should be italized.
Answer: Text is revised as directed.
6. Page 11: Is it required for the abbreviation MCE?
Answer: Text is revised as directed.
7. (References) titles of the cited articles should carefully be corrected: bad use of capitals.
Answer: Text is revised as directed.
8. Figure legends should be shortened by removing redundant statements that were already stated in the METHODS section.
Answer: Text is revised as directed.
9. (Fig. 2A, B, C, D): beta and gamma should not be separated
Answer: Figure caption is revised as directed.
10. (Fig. 4B) CAL>CAF?
Answer: Text (figure) is revised as directed.

11. (All figures) units should be separated from values (e.g. 10 ug/ml).
Answer: Figure caption is revised as directed.

Reviewer 2#
Major comments.
1. The authors should show the detail components of extract of CAF using in this experiments.
Answer: The CAF extracts were supplied by Dr. Shin and Kim’s Labs and it was citated in this manuscript reference (21, 22). Kim’s paper has been published in last Month. Analysis of the individual components of CAL extracts by HPLC were conducted at Dr. Shin and Kim’s Labs of Gyeongsang National University, and CAL consists of many flavonoids, naringin, hesperidin, poncirin, isosiennsetin, hexamethoxyflavone, sineesytin, hexamethoxyflavone, tetramthnl-o-isoscutellaeein, nobiletin, heptamethoxyflavone, 3-hydoxynobiletin, tangeretin, hydroxypentamethoxyflavone, and hexamethoxyflavone.

2. There are many poorly written sentences and inappropriate use of word or phrases. For example, Dulbecco’s modified eagle medium (DMEM) high glucose with 10% calf serum at 37C in a humidified atmosphere of 5% CO2. (page 4, line 8-9) etc.
Answer: Text is revised as directed.

3. Some unsuitable references were cited. The authors should carefully check them again.
Answer: Text is revised as directed.

4. The authors should add the figure of the mechanisms of CAF.
Answer: We would like to thank the reviewer’s comments, and we think to further experiments to illustrate the mechanism of CAF.

5. In figure 4B, the authors should add the results of DMII.
Answer: Text is revised as directed.

Minor comment
1. The format of some references (22 and 23) is not collect.
Answer: We would like to thank the reviewer’s comments. Text is revised as directed.

Reviewer 3#
MAJOR COMPULSORY REVISIONS:
1. The title of the manuscript should use the appropriate form of the verb "to inhibit" for the third person plural ("Citrus aurantium flavonoids"). On the other
hand, terms adipogenesis and adipose differentiation are synonymous, so that their simultaneous use in the same sentence is redundant and should be avoided. Since only a model of adipogenesis was used, the title should specify that studied compounds inhibit 3T3 adipogenesis. Finally, it is an accepted convention to write scientific names in italics.

Answer: We would like to thank the reviewer for pointing out this insufficiency. The text has been revised to point out the issues and the manuscript was changed according to the reviewer comments.

2. Authors should specify the source of 3T3-L1 cells used. They should also specify the source and date of collection of plant material, its identification by an expert in botany, and if a backup copy of plant material exist in a recognized herbarium. Authors should also specify the part of Citrus aurantium plant used in the study, how plant material was processed previously to methanol extraction, and the ratio of plant material weight/solvent volume used for the methanol extraction.

Answer: The CAF extracts were supplied by Dr. Shin and Kim’s Labs and it was citated in this manuscript reference (21, 22). Kim’s paper has been published in last Month. Analysis of the individual components of CAL extracts by HPLC were conducted at Dr. Shin and Kim’s Labs of Gyeongsang National University, and CAL consists of many flavonoids, naringin, hesperidin, poncirin, isosiennsetin, hexamethoxyflavone, sineesytin, hexamethoxyflavone, tetramrthnl-o-isoscutellaein, nobiletin, heptamethoxyflavone, 3-hydoxynobiletin, tangeretin, hydroxypentamethoxyflavone, and hexamethoxyflavone.