Author’s response to reviews

Title: Differences in anti-inflammatory effect of immature and mature of Rubus coreanus fruits on LPS-induced Raw 264.7 macrophages via NF-κB signal pathways

Authors:

Ji Lee (ljy341@chungbuk.ac.kr)
Jung Park (yong0433@korea.kr)
Gwi Jang (janggy@korea.kr)
Hyung Don Kim (radioleg@korea.kr)
Young Lee (youngseoblee@korea.kr)
Kyung Hye Seo (seokh@korea.kr)

Version: 1 Date: 12 Dec 2018

Author’s response to reviews:

Dear Editor

I wish to submit an research article, titled “Differences in anti-inflammatory effect of immature and mature of Rubus coreanus fruits on LPS-induced Raw 264.7 macrophages via NF-κB signal pathways.” for consideration for publication in BMC Complementery and Alternative Medicine.

Reviewer reports:

Qiong Liu (Reviewer 1):

About research content

1. The authors make no mention of choosing the RAW264.7 cells in their manuscript.

   - We added in ‘Discussion’, page12 line 22-34, page 13 in line 2-3 and page 14 in line 2-4.

RF is well known to have various pharmacological effects including anticancer and anti-inflammatory [1-5], which are attributed to the phenolic compound, anthocyanin, and EA content.
However, the anti-inflammatory effects of RF on LPS-induced RAW264.7 cells related to fruit maturity and differences in extraction has not been examined. Macrophage, Raw 264.7, was have major function such as the initiation, maintenance, and resolution in the inflammatory precess [9].

2. What rationale is there for the concentrations of ethanol(0, 30, and 70%), why not 100%?

- We added in ‘Discussion’, Page13 in line 6-10.

TPC and TFC was higher 75% ethanol extraction of MRF than 25%, 50% and 100% ethanol extraction [10] and anti-oxidant effect, also, 75% ethanol extraction of MRF and IRF better than 25%, 50% and 100% ethanol extraction in liver cell and DPPH radical scavenging activity [10, 11]. In addition, 30% ethanol extraction of IRF has beneficial effect osteoporotic by pro-inflammation [12]. So, water, 30% and 70% ethanol were selected for extraction solvents. Thus, our study reported the first comparison of the anti-inflammatory effect of different solvent extracts of MRF and IRF on LPS-induced RAW264.7 cells.

3. In the discussion, lines 21-22, how can this conclusion be drawn?

- In the discussion, page13 line 18-22, we described that EA (ellagic acid) had anti-inflammatory activities and included reference to it.

EA is known to be a standard phenolic compound in RF and, therefore, EA was selected as a standard component for the analysis. EA has potential activities such as anti-inflammatory and anticoagulatory in cardiac tissue of diabetic mice and UV-B irradiation-induced inflammation [13, 14].

4. Please check if the result corresponds to the corresponding picture.

- We changed the figures number.

About the result,

1. First result, There is no description of the sample size and analysis method of the data, and no corresponding picture or table has been visually presented to the reader.
- ‘Materials and methods’ were added to the method. Results are not shown in the table because all results are described in ‘results’.

The samples were measured each dried weight and freeze-dried extracts weight for yield. The weight of dried samples (Wsample) and the freeze-dried extracts (Wextracts) were weighed, and the yield was calculated by the following equation:

\[
\text{Yield (\%) = } \frac{\text{Wextracts (g)}}{\text{Wsample(g)}} * 100
\]

2. Second result, (1) The comparison of IC50 in IRF and MRF should be performed at the same ethanol extraction concentration when describing the results. (2) Where does the "P<0.05" on line 16 in the result come from, which statistical method is used? There is no relevant explanation. (3) What is the letter mean after each IC50 value in Table 2?

- (1): We wanted to compare the IC50 values in all extracts (regardless of solvent). (2): Statistical methods are described at the bottom of the table. (3): The meaning of each character is also described at the bottom of the table.

3. Third result, (1) The description of the results in lines 21-22, "The cell viability values were > 90% following exposure to up to 200 μg/mL of the extracts compared to the control (LPS treated only, Fig. 1a)" is too general. Significantly down-regulated in the IRF group at 0%, 25μg/mL and 30%, 50μg/mL, are these more than 90%? (2) It is very strange that we did not find where is the "Fig. 1b". May be it's Fig 2, please correct it.

- (1): Since all the extracts showed cell viability of more than 90%, up to 200 μg / mL, they were described as page 10 in line 1-4. (2): Fig.1a and 1b were modified to Fig.1 and Fig.2.

4. Fourth result, (1) Pictures and legends do not match the results. (2) There were no RT-PCR results for related pro-inflammatory cytokines and no statistical results.

- (1): Fig.2 was modified to Fig.3. (2): Statistical results were shown in Fig.3.

5. Fifth result, The results in the article do not match the pictures, there is no corresponding statistical analysis, and no relevant conclusions can be drawn.

- Fig.3 was modified to Fig.4 and statistical results were shown in Fig.4.
6. Sixth result, The picture does not match the result, there is no corresponding statistical analysis, and the legend cannot clearly describe what is being done.

- Fig.4 was modified to Fig.5 and analysis results are shown in Fig. 5.

Yogesh A. Kulkarni, Ph.D. (Reviewer 2): Comments

1. Authors should add authentication details for plant material.

- We added details for plant material in material section of “Material and Method” on page 5 in line 10-14.

“R. coreanus grown for 30 and 45-70 days post-bloom as the IRF and MRF, respectively were collected from Gwang Yang in South Korea in 2017. A code and production income sales report number of the plant material (09-01-0026 and 09-004-2005-1) was deposited at the National Forest Seed and Variety Center and identified [15]. In addition, the voucher specimen (NIBRVP0000180126) was deposited at National institute of biological resources [16].”

2. Authors should justify selection of 30, and 70% ethanol extract for study.. Why not 50%, 40%..etc

- We added in ‘Discussion’, page 13 in line 2-6.

“TPC and TFC was higher 75% ethanol extraction of MRF than 25%, 50% and 100% ethanol extraction [10] and anti-oxidant effect, also, 75% ethanol extraction of MRF and IRF better than 25%, 50% and 100% ethanol extraction in liver cell and DPPH radical scavenging activity [10, 11]. In addition, 30% ethanol extraction of IRF has beneficial effect osteoporotic by pro-inflammation [12]. So, water, 30% and 70% ethanol were selected for extraction solvents. Thus, our study reported the first comparison of the anti-inflammatory effect of different solvent extracts of MRF and IRF on LPS-induced RAW264.7 cells.”

3. Authors should provide make of all antibodies used for western blotting.

- All antibodies are written on page 9 in line 1-3.
“Phospho Nf-κB p-65 (p-p65), phospho-IκB-α (p- IκB-α), Cox-2, iNOS and β-actin were used for primary antibodies. All antibodies were purchased by cell-signalling (USA).”