Author’s response to reviews

Title: Isoferulic acid prevents methylglyoxal-induced protein glycation and DNA damage by free radical scavenging activity

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Title: Isoferulic acid prevents methylglyoxal-induced protein glycation and DNA damage by free radical scavenging activity

MS: BCAM-D-15-00002
Author: Meeprom et al.

Thank you for consideration of our manuscript for publication in your journal. We greatly appreciate the time both you and the referees have put into offering feedback for improving our manuscript. We have reviewed the above manuscript according to your reviewer’s comments.

Reviewer #1: There are a few language corrections as well as a few comments that need to be addressed.

The statistical test is inappropriate and a non-parametric test should be applied. The results of the post-hoc test is not shown anywhere and can be left out of the methods description.

Re: We have already checked the normality of group data, therefore, followed by One-way ANOVA. One way ANOVA We corrected the sentence according to the comments. “All data are presented as means ± SEM. In the experiment of MG-derived AGEs, two-way ANOVA was evaluated for the significant differences among groups. Other experiments were analyzed the significant differences by one-way ANOVA. Duncan’s post-hoc test was used to examine
differences among groups. A p-value < 0.05 was considered statistically significant. In the experiments of MG-derived AGEs, two-way ANOVA was applied for this section because there was another factor of time involving in this experiments. Post hoc test has been clarified in this section.

Comment from the track changes: what about the non-fluorescent AGE? This also means only two measurements. What was it compared to and were there any blank assays to check if natural ageing without methylglyoxal resulted in increased fluorescence?

Re: We added the sentence to clarify the detail of calculation on fluorescent MG-derived AGEs below:

% Inhibition = Fluorescent intensity of (BSA/MG - BSA/MG/IFA) x 100 Fluorescent intensity of BSA/MG

Meanwhile, non-fluorescent N-CML (one type of non-fluorescent AGEs) was detected by using commercial ELISA. In the results, it was expressed as ng/ml in Table 1, fluorescent absorption would not interfere this measurement.

In this section, fluorescent MG-derived AGEs was replaced to specifically identified products from the action of MG. The non-fluorescent εN-CML was replaced from non-fluorescent AGEs.

Comment from the track changes: This sentence should rather be in your discussion “The protein carbonyl concentration is a marker of protein oxidation.”

Re: It has been removed from this section.

Comment from the track changes:

1. insert the words "in the intensity" of the OC band.

2. Move this reference to the end of first sentence and remove next two references to the same source.

3. Reword to read "Previous studies reported ....".

4. insert word "of" between scavenging and superoxide.

5. insert the excitation and emission wavelengths used.

6. insert the words "as measured by cytochrome c reduction"

Re: We followed corrections according to the comments.

The figure legends should have more detail. In particular the gel images should carry at least the treatment to make it easier to interpret the results seen. The graphs of the band densities appear quite different from what is seen visually and this should be highlighted. The way the band
densities were determined should be included as the settings used in the software can make a big difference.

Comment from the track changes: It would be more meaningful to have the treatment indicated for each lane of these three figures instead of the lane number

Re: We have changed the figure and showed the detail of experiment in the figure legends and the band detail of each lane.

Are the incubation times appropriate to make physiological conclusions?

Re: Actually, time of incubation are consistent with the duration of MG action. It is very fast to produce free radicals after incubation. We also measured the MG action for 1-2 weeks demonstrating the normal glycation process in blood circulation. The glycation process can take place at the beginning of reaction or more than 1 week. For example, there were several publications regarding investigation the glycation 1 to 4 weeks using reducing sugars.

Ref.


There is mention of ELISA but there are no results shown for that assay? Rather take out the mention of the assay.

Re: The results showed in the section of non-fluorescent N-CML. This measurement was used ELISA representing in the materials and methods.

Comment from the track changes: The ratio of 2MQ/5MQ should be added to each chromatogram to highlight the differences. The legend should also include a statement with respect to the observed result that only the MG + AG showed any scavenging activity.

Re: the ratio of 2MQ /5 MGQ could not add to the figures because this value must take the calculation with the standard curve in order to express as MG concentrations. To clearly clarify of the calculation, we have added the sentence in the materials and methods. The 2- and 5-MQ was monitored at 315 nm. Peak integrality ratios of 2-MQ to 5-MQ were used for quantitative analysis. The amount of MG was calculated by using the standard curve of 2-MQ/5-MQ. The percentage of MG reduction was calculated using the equation below.

\[
\% \text{ Reduction} = \frac{\text{Amount of (MG in control} - \text{MG in test compound})}{\text{Amount of MG in control}} \times 100
\]
The sentence regarding the effect of AG on the MG-trapping ability has been already referred to the results.

We hope that you will now find the revised manuscript acceptable for publication in BMC Complementary and Alternative Medicine. We have included a manuscript with highlighted changes for you to see exactly what has been revised in the resubmission.

Sincerely,

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