Author's response to reviews

Title: Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: A randomized, double-blind, placebo-controlled trial

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Author's response to reviews: see over
Dear Nehme Gabriel

We are very happy to resubmit our revised version. We sincerely appreciate the time spent in reviewing this manuscript and your advice to improve it. Please, see below our answers to your queries and comments. We also marked the corrected and revised parts of the text in red. We hope that you find them satisfactory. The principle author and all co-authors have read, and approved the submission of the revised version of manuscript; the material is an original research, has not been published and is not being considered for publication elsewhere, in whole or in part, in any language, except as an abstract. And there will not be any potential conflict of interest.

**Full title:** Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants

**- Corresponding author & Address:** Jong Ho Lee, Ph.D., R.D.
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Thank you very much for your time.

Best regards,

**Jong Ho Lee, Ph.D., R.D.**
Full professor,
Dept. of Food and Nutrition,
College of Human Ecology, Yonsei University
Response to Reviewers

MS: 6821581306442202

Title: Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: A randomized, double-blind, placebo-controlled trial

Dear Reviewer

In accordance with your advices, we made answers.
We hope that they would be appropriated.

Sincerely yours,

Jong Ho Lee, Ph.D., R.D.
Full professor,
Dept. of Food & Nutrition, College of Ecology
Yonsei University, Seoul, Korea
**Reviewer 1**

**Reviewer’s comments**

This study investigated the effect of Korean red ginseng (KRG) on oxidative damage (DNA and antioxidative enzyme activity and lipid peroxidation) in healthy subjects. The presented results showed an increase of SOD activity after 8 weeks of KRG supplementation, plasma Gpx and catalase activities were also increased. The DNA damage, as measured by DNA tail and tail and oxidized LDL were significantly reduced after KRG. The author concluded that KRG supplementation might attenuate lymphocyte DNA and LDL oxidation by upregulating antioxidants enzyme activity.

**Major points:**

The SOD and catalase activities were measured in the serum. However, both SOD and catalase activities in the serum are very low when compared to the activity of these enzymes in the plasma membrane. So it is not clear why the authors preferred to measure the SOD and catalase activities in the serum instead of plasma membrane of cells such as erythrocytes?

*Answer*: In accordance with your advice, we additionally measured SOD and catalase activity on erythrocytes (RBC). We found that baseline values of SOD or catalase activity among the 3 groups were not significantly different, but we found significant differences in the net differences, particularly of SOD activity between placebo and high-dose group, which maintained after the adjustment (please see the Table below). We added these results in the text.

<table>
<thead>
<tr>
<th></th>
<th>placebo</th>
<th>Low-dose</th>
<th>High-dose</th>
<th>Adjusted p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC-SOD Baseline</td>
<td>44.1±3.14</td>
<td>46.0±2.93</td>
<td>42.3±2.99</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-3.58±2.39</td>
<td>-0.07±1.81</td>
<td>3.50±1.95†</td>
<td>0.030</td>
</tr>
<tr>
<td>RBC-Catalse Baseline</td>
<td>231.1±1.91</td>
<td>224.3±3.48</td>
<td>223.7±2.78</td>
<td></td>
</tr>
<tr>
<td>Change</td>
<td>-6.74±2.94</td>
<td>1.21±3.45</td>
<td>4.46±2.55†</td>
<td>0.120</td>
</tr>
</tbody>
</table>

*Tested by analysis of covariance (ANCOVA) using general linear model (GLM) with adjustment for age, sex, BMI, smoking, drinking, systolic BP, diastolic BP, and baseline value

†P<0.05 compared with placebo group
It is not clear if the increase of the SOD and CAT activities is due to an increase of SOD and CAT proteins concentration or to an increase of the specific activities of these two enzymes.

*Answer*: As you commented, it would be better for the explanation if both enzyme activity and protein mass were measured. However, we only measured the enzyme activity, because we could not get the assay kit for enzyme protein mass. Instead, our results may be partly explained by the report of Chang et al showing that Rb2 (Panaxadiol Ginsenosides) extracted from Panax ginseng increased transcriptional activation of SOD and catalase genes. That is, it is assumed that Rb2 induces the production of enzyme (protein mass) which may increase the enzyme activity. But to make it sure and confirmed, we need to measure the mass itself or the gene and protein expression (i.e. transcriptional and posttranslational levels). We mentioned it in the limitation part of the text.

The author measured oxidative damage to DNA and to lipid (oxidized LDL). However a measurement of oxidative damage to protein fraction (measurement of carbonyl groups) would have been very relevant to determine whether the increase of the SOD activity is due to a reduction of oxidative damage to protein or to an increase of the SOD synthesis.

*Answer*: As you pointed out, and as we stated above, we may need to measure the enzyme activity and protein mass to make it clarified. Therefore, we mentioned it in the limitation part of the text.

**Table 3: Gpx and Cat activities are low in the low and high-dose groups at baseline when compared to placebo group?**

*Answer*: There are no significant differences among the low and high-dose groups and placebo group at baseline. Also we performed general linear model for the comparison of net change values among the 3 groups with adjustment for baseline value.

[Minors]

line 297, add a reference for the comparison between Korean ginseng and American ginseng

*Answer*: As you pointed out, we added the reference.
Reviewer 2

Reviewer’s comments

The authors provide an article on KGR consumption in healthy humans. The work could be useful, however it is presented in a fragmentary way and appears confuse: there are a lot of concepts without a linkage and an explanation. The paper will be published after major and deep revisions

Abstract

The background of the abstract should be improved by focusing the attention on the importance and role of KRG supplementation on oxidative stress in healthy humans. In the abstract at results section, delete the term … after adjustment…..(line 43,46 and 48)

Answer : As you pointed out, we deleted the term.

Introduction

The background should be improvable by focusing on relevant information that strictly reflects the main goal of the submitted manuscript. In particular: Add current references regarding ginseng composition and supplementation even if the mechanism for ginseng’s health-promoting effects is unknown. Move from discussion line 295 to 304 in this section. In the same way the controversies regarding supplemented dose should be reported. In addition the section from line 62 to 63 appears without a linkage and should be better organized. The manuscript would be much improved if the background would provide a brief but referenced overview of the literature data available.

Answer: In accordance with your advices, we rearranged the background part.

Methods

Subjects: From Results move line 239 to 242 after line 99 in this section

Answer : As you advised, we re-arranged the part.

Test capsule and study design: Move line 111 to 116 at the beginning of this section and then add the study design including from line 100 to 110.

Answer : As you advised, we re-arranged this section.

In addition the authors should clarify and better explain how many capsules were
consumed from subjects: 10 capsules in total per day or 10 capsules after each meal?

Answer: As you advised, we clarified the sentence.

“All subjects were asked to take 10 capsules in total per day, immediately after any of main meals (for example: 3 capsules after breakfast, 3 capsules after lunch, 4 capsules after dinner)’’.

It should be useful to include the total daily supplementation in L and H dosage.

Answer: the total numbers of capsules were the same (10 capsules/d) for all the 3 groups. But the amount of red ginseng were different (0.3g/d or 0.6g/d).

Move Safety parameters before Serum lipid profile and fasting glucose

Answer: As you advised, we moved the section.

Results
Start from line 244.

Answer: As you advised, we corrected it.

The quality of results could be improved showing a figure of Comet assay if the authors agree.

Answer: As you advised, we presented the image of comment assay in the figure.

Discussion
I would suggest the authors to interpret and discuss the described results with a proper embedding in the literature available on the topic. The discussion should totally rewritten by focusing the attention on the obtained results that should be appropriately discussed. In particular: From line to 289 to 294 were summarized the results observed in this study, so move the lines in the Conclusion. Start the discussion from line 305 and even if mechanisms of action was not addressed in the study design the authors should be formulated some hypothesis of their achievements.

Answer: As you advised, we rearranged and rewrote this part.

Line 309:……..supplementation (6g)…….specify per day or 8-week?

Answer: We made this part clear.

“In the present study, 8-week KRG supplementation (6g/day)…”
Line 337-339: Why the study would be useful to design a cranberry supplementation? I don’t understand the rationale and the purpose of the sentence.

Answer: I am sorry for making you confused. It was mistake. I corrected it.

It is not clear if the increase of the SOD and CAT activities is due to an increase of SOD and CAT proteins concentration or to an increase of the specific activities of these two enzymes.

Answer: As you pointed out, it would be better for the explanation if both enzyme activity and protein mass were measured. However, we only measured the enzyme activity, because we could not get the assay kit for enzyme protein mass. Instead, our results may be partly explained by the report of Chang et al showing that Rb2 (Panaxadiol Ginsenosides) extracted from Panax ginseng increased transcriptional activation of SOD and CL genes. That is, it is assumed that Rb2 induces the production of enzyme (protein mass) which may increase the enzyme activity. But to make it sure and confirmed, we need to measure the mass itself or the gene and protein expression (i.e. transcriptional and posttranslational levels). We mentioned it in the limitation part of the text.

Conclusion

Conclusions should be improved by highlighting the achievements and outcomes

Answer: As you advised, we rearranged and rewrote this part.

Figure 1

Check the reported significance at level ††

Answer: We checked and corrected it.

“†P<0.05, ††P<0.01, †††P<0.001 compared with placebo group.”
Reviewer 3

Reviewer’s comments

The manuscript entitled “Beneficial effects of Korean red ginseng on lymphocyte DNA damage, antioxidant enzyme activity, and LDL oxidation in healthy participants: A randomized, double-blind, placebo-controlled trial” aims to evaluate effect of Korean red Ginseng extract on oxidative stress in healthy patients. While the manuscript draws attention to growing need of evaluation of clinical efficacy of widely consumed natural health products, there are some concerns discussed below that need to be addressed.

Major:

The design of the clinical trial failed to set out primary and secondary outcome measures. The authors proposed an overly large number of outcome measures (blood pressure, BMI, Total cholesterol, LDL, triglycerides, DNA damage, plasma SOD activity, plasma GPx, plasma catalase activity, oxidized LDL, urinary 8-epi-PGF, BUN, creatinine, CBC, AST and ALT). In addition power analysis is not provided (what parameter is the power based on?). The rationale for certain measurements and their significance in the context of the trial is also missing.

Answer: We selected the biomarkers and determined the number of subjects by following the criteria for the test of antioxidant/oxidative effect recommended by Korea Food and Drug administration (KFDA). According to the KFDA guideline, the first biomarker for the test of antioxidant/oxidative effect is DNA damage (i.e. tail moment and tail length) measured by comet assay and the second ones are antioxidant enzymes. If the net difference of the DNA damage before and after the intervention in the test group is at least 10% different from that in the placebo, we determined it is statistically significant different. Based on this concept, we calculated the power and the number of subjects following the formula.

Minor:

Abstract: 1. The terms SOD, GPx are not defined in the abstract. Abstract should be self-standing.

Answer: As you pointed out, we corrected it.

Background:

1. What is the reason the authors are evaluating healthy individuals instead of those
with underlying pathology? The rationale should be provided.

Answer: Recently, the interest in red ginseng has been growing. It is thought to be one of foods which can be consumed in daily lives in Korea., but one of foods which can be consumed in daily lives in Korea, and there’s no report on the effect of RG in healthy individual. In accordance with your comment, we clarified this part in the text

2. The background should differentiate pre-clinical and clinical research when describing that ginseng “is known to exert” a number of effects.

Answer: As you advised, we corrected it.

“Therefore, a number of pre-clinical studies (10-12) and clinical study (13) have reported medicinal benefits of Korean red ginseng (KRG) including antioxidant, antitumor, antimutagenic, and immunomodulatory actions,…”

3. Background focuses on listing effects of ginseng rather than provide brief general overview of present literature and more channeled rationale for study.

Answer: Following your advice, we explained this part more in detailed.

Test Capsule and Study design

1. What is the quantity of placebo used in the trial?

Answer: The placebo is KRG-flavored 0.3g capsule containing corn starch. We stated that as below.

“Identical-looking capsules contained red ginseng (low dose; 0.3 g, high dose; 0.6 g) or placebo (0.3g of KRG-flavored capsule containing corn starch).”

2. Were subjects who only used cigarettes or alcohol included in the trial? The statement should read: use of cigarettes and alcohol was not an exclusion criteria.

Answer: We are sorry for making you confused. We included subjects who either used cigarettes or consumed alcohol (or both).

3. Are ten capsules taken at once after a meal of choice or spread out between 3 meals? This may introduce a confounding variable.

Answer: We are sorry for making you confused again. We clarified this part.

All subjects were asked to take 10 capsules in total per day, immediately after any of main meals (for example: 3 capsules after breakfast, 3 capsules after lunch, 4 capsules after dinner)
Assessment of dietary intake

1. What is the reason that participants continue their usual diet for only one week? Should it be for the duration of the study or during competion of a 3-day food record?

Answer: To make it clear, we re-arrange the explanation this part.

All the subjects were given written and verbal instructions by a registered dietitian on completion of a 3-day (2 week days and 1 weekend) dietary record every 4 weeks. On the sheet, subjects were instructed to record the amount of foods before ingestion and any remaining after ingestion by weighing the foods. A week before the start of the study, all the participants were advised to continue their usual diet for a week and instructed to complete a 3-day dietary record as the baseline measurement, which were compared with the record obtained by 24-hour recall methods at previous visit in order to check their records. Participants were also instructed to record their physical activity diary for 24 hours every 4 weeks. To check participants’ compliance during the whole study period, the dietitian interviewed them biweekly by telephone. During the study period, all participants were also encouraged to maintain their usual lifestyle.

Anthropometric parameters, blood pressure

1. It would have been more accurate to measure blood pressure in triplicate. 20-min of rest is usually not necessary and 10 minutes would suffice.

Answer: We explained it more in detailed, subjects took 20-min of rest and were measured for blood pressure in triplicate for accuracy.

2. Study measurements sections are too detailed in comparison to the protocol patients followed. The protocol does not state when the measurements are obtained, how many visits did participants have, when were each of the measurements taken.

Answer: Participants were asked to visit 4 times (a week before, 0, 4, 8 week). Blood sample were measured at 0 and 8 week and diet and capsule intakes were checked 0, 4, 8 weeks. We added this sentence and rearranged this part.

Results/Discussion:

1. It is peculiar that none of the 69 subjects reported any adverse events for the duration
of 8 weeks. The authors only reported on serious adverse events. However, nausea, hot flush and others listed are not to be considered as a serious adverse events (SAEs) as per ICH/GCP guidelines.

*Answer:* As you commented, we revised and corrected the explanation.

2. Figure 1 does not have a self-standing description accompanying the graph.

*Answer:* We added it.

3. Discussion describes correlation between changes in plasma LDL and decrease in oxidative damage and urinary 8-epi-PGF2α; however, such correlation analyses have not been described in statistical methods section or results section. Is this correlation statistically significant?

*Answer:* Yes, it is statistically significant. We added the results section.

**Relationship between changes in oxidative stress markers and catalase activity:** Among all subjects, changes in oxidized LDL from baseline correlated positively with changes in urinary 8-epi-PGF$_{2α}$ ($r=0.305$, $P=0.030$), correlated negatively with changes in catalase activity ($r=-0.419$, $P=0.002$), and appeared to correlate positively with changes in DNA tail moment ($r=0.231$, $P=0.096$), but this correlation was not significant. After adjusting for sex and age, changes in oxidized LDL correlated positively with both changes in urinary 8-epi-PGF$_{2α}$ ($r=0.332$, $P=0.026$) and changes in DNA tail moment ($r=0.295$, $P=0.049$), and negatively with changes in catalase activity ($r=-0.401$, $P=0.006$).

4. The discussion drawing parallel between Korean red ginseng and American ginseng is not placed in the context of the trial and does not substantiate hypothesis in any way. It may be more appropriate to discuss differences between white and red varieties of Panax ginseng spp. in relation to oxidative stress.

*Answer:* we are sorry for making the reviewer confused with unclear explanation. We re-explained this part, and we also unified the term white ginseng instead of American ginseng.