Author's response to reviews

Title: Hepatoprotective effect of curcumin and alpha-tocopherol against cisplatin-induced oxidative stress

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Author's response to reviews: see over
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Version: 2 Date: 7 February 2014

Author's response to reviews: see over
Reviewer's report

Title: Hepatoprotective effect of curcumin and alpha-tocopherol against cisplatin-induced oxidative stress

Version: 2 Date: 4 February 2014

Reviewer: Somchai Pinlaor

Reviewer's report:

Major comments

1) Why the author pre-treated with single dose of α-tocopherol (250 mg/kg b.w.), curcumin (200 mg/kg b.w.) and α-tocopherol with curcumin, respectively, via i.p. route for 24 h prior the administration of cisplatin? Timing, route and dose of α-tocopherol and curcumin are necessary or cited reference.
   • The rationale behind the selected dose based on previous studies including Palipoch et al., 2013 and Palipoch et al., 2013 was cited (line 127)

2) Why animal were sacrificed after 72 h of first injection? Is this time show the highest activity for the pharmacokinetic of curcumin and α-tocopherol on hepatoprotective effect?
   • No, this time show high levels of liver injury markers the highest level of AST and high level of ALT) of cisplatin-treated group according to Palipoch and Punsawad, 2013)

3) Background should be re-written because it is not clear whether for the major problem to study between oxidative stress and cisplatin. The author can start with the major problem first, such as cisplatin-induced adverse effect on chemotherapy treatment of many cancers. Although the mechanism of cisplatin-induced adverse effect is still unclear; however, many evidences showed that its hepatotoxicity is believed via free radical generation-mediated oxidative stress dependent mechanism.
   • Done

4) Discussion should be re-written based on the results and explained the results. Why curcumin and α-tocopherol have hepatoprotective effect in cisplatin-induced oxidative stress in rat? Why the combination can enhance this hepatoprotective effect?
   • Done

Minor comments

1) The detail of curcumin such as number of catalogue and purity are necessary to show. Because of the different purity of curcumin have different protective effect on oxidative stress markers and antioxidant enzyme activity.
   • Add number of catalogue and purity of cisplatin and curcumin: cis-Diammineplatinum (II) dichloride (product number: 479306, purity ≥ 99.9%) and curcumin from Curcuma longa (product number: c1386, purity ≥ 65%) (line 101-102)

2) Also detail of α-tocopherol is necessary to mention.
• Add number of catalogue and purity of α-tocopherol: α-tocopherol (product number: 258024, purity ≥ 95.5%) (line 103)

3) Liver function test includes not only ALT and AST, Fig.1 should change to use ALT and AST directly or liver injury markers (ALT and AST).
  • Done

4) Mitochondria SOD is SOD2 but not SOD1, page 15, line 334.
  • Done (line 302)

5) The term oxidative stress is an imbalance between oxidant and antioxidant, this term should be carefully used throughout ms.
  • Done (line 69)

6) How many experiments were performed for each technique, duplicate or triplicate?
  • Duplicated

7) Fig 4 and Fig5 can be combined because the author used the same technique.
  • Done

8) RT-PCR is semi-quantitative technique, especially loading of PCR product. This should state in the method whether “all sample was equally loaded on 2% gel”
  • Done (line 209)

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published
  • Done

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report

Title: Hepatoprotective effect of curcumin and alpha-tocopherol against cisplatin-induced oxidative stress

Version: 2 Date: 7 February 2014

Reviewer: George Asare

Reviewer's report:

REVIEWER’S COMMENTS

MAJOR ISSUES

1. What was the rationale behind selecting 200 mg/kg b.wt curcumin when all others are 250 mg/kg b. wt.
   • The rationale behind the selected dose based on previous studies including Palipoch et al., 2013

2. What is the therapeutic dose of cisplatin in cancer treatment and what was the rationale behind the selected dose?
   • Therapeutic dose is 20 mg/m2/day for 5 days, 20-30 mg weekly for 3-4 weeks, or 100 mg/m2 given once every 4 weeks.
   • The rationale behind the selected dose based on my previous study, Palipoch and Punsawad, 2013 and Palipoch et al., 2013 which enough to induce hepatic injury

3. Cisplatin is used in the treatment of cancers which is a chronic condition. Why was an acute study designed for a chronic condition?
   • Although cisplatin is used in the treatment of a chronic condition, however its clinical toxicities can occur in the first 24-96 h after administration

4. For animal studies, the standard statistics should be mean ± SEM and not mean ± SD. The statistics should be done again.
   • Done (line 214)

MINOR ISSUES

Line 51……may be involved in abrogate oxidative….change to may be involved in abrogating.
   • Done (line 52)

Line 65. …..in treatment of various cancers…change to…..in the treatment of various cancers.
   • Done (line 65)

Line 67…… however, many evidences showed ….change to……however several evidence have shown…..
   • Done (line 67)
Line 74. ..which has ability to generate…change to….which has the ability to generate.
  • Done (line 74)

Line 84-85.. Change to …leads to oxidative stress by increasing lipid peroxidation as demonstrated in mice.
  • Done (line 84-85)

Line 89/90.. Change to….which has been demonstrated to possess antioxidant activity in vivo.
  • Done (line 89-90)

Line 94/95… we aimed to gain insight and to understand the biochemical,…..change to….
The study aimed at gaining insight into the understanding of the biochemical,…… .
  • Done (line 94-95)

Line 104. …Inc (………………, USA). Always state Town and Country. .
  • Done (line 104)

Line 112/113….. were conducted to the Guide for the Care and…..change to…were conducted according to the Guidelines for the Care and.
  • Done (line 113-114)

Line 117….. before the researcher performed….change to……before the commencement of…….
  • Done (line 118)

Line 122….. Change to were treated with a single dose.
  • Done

Line 128. After opening the abdominal cavity, the liver was collected and……change to….After opening the abdominal cavity, the liver was harvested.
  • Done (line 130/131)

Line 152. Was homogenized to……..change to…..was homogenized to give a final concentration of…….
  • Done (line 153)

Line 155. Town and Country of kit. .
  • Done (line 156)

Line 236. cisplatin significantly demonstrated the reduction….change to cisplatin significantly led to the reduction (Change similar phrases throughout) .
  • Done (line 237)

Line 265/266. The up-regulation of NADPH oxidase gene expression indicates the molecular alterations. REPHRASE.
  • Done (line 165-166), change to “The molecular alteration indicates the up-regulation of NADPH oxidase gene expression.”
271/272. The inefficiency and the insufficiency of antioxidant defense system were concerned in various pathological conditions. STATEMENT NOT CLEAR.

- Done (line 271-272), change to “Reduction of antioxidant defense system was concerned in various pathological conditions”

Line 280. Delete….been…….
- Done (line 280)

Line 295. The pre-treatment with combined curcumin and #-tocopherol illustrated the….change to……Pre-treatment with combined curcumin and #-tocopherol led to the.
- Done (line 295)

Line 310. …involved in abrogate oxidative stress…..change to……involved in the abrogation of oxidative stress
- Done (line 310)

Line 322. ANOVA, One-way analysis of variance. ANOVA mean….analysis of variance.
- Done (line 323)

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests
Reviewer's report

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**Version:** 2 **Date:** 7 February 2014

**Reviewer:** P. S. Bedi

**Reviewer's report:**

**Major Compulsory Revisions:**

1. No compulsory revisions have been done as recommended
   - OK
2. Background needs to be given in brief.
   - Done
3. No additional experiments are required.
   - OK
4. Statistical data has not been given. It was asked in the earlier review as well.
   - OK

**Earlier review**

1. Major compulsory revision

Data should be presented in the table form so that statistical analysis of the data shall be checked. It gives more authenticity to your work.
   - Data were presented in the graph form, but we added the significant p-value comparing between group

2. Minor essential revision

No need to give reference after each disease in background, the same may be given in the last of the sentence for eg. Various diseases including liver, neurodegenerative, cardiovascular, lungs and kidney [1-5].
   - Done

3. Discretionary revision

Background should be brief. Previous studies reported in the text related to various parameters studied may be used in discussion.
   - Done

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
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