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
A cover letter giving a point-by-point response to the concerns

Referee 1

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. (add references: Palipoch et al., 2013)

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. *(Re-written)*

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? *(add more informations)*

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%))

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%))

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). *(change to AST and ALT)*

4) Mitochondria SOD is SOD2 but not SOD1, page 15, line 334. *(edit to SOD2)*
5) The term oxidative stress is an imbalance between oxidant and antioxidant, this term should be carefully used throughout ms. (OK)
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. (combined Fig 4 and Fig 5)
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” (state “all sample was equally loaded on 2% gel” in the method)

Referee 3
Major Compulsory Revisions
1. Abstract. How many rats per group? (n=5)
   Experimental set-up
   2. Did the animals feed and drink freely?? (Yes)
   3. How many rats per group?? (n=5)
   4. line 98. Additionally, α-tocopherol is the most biologically active form of vitamin E. NOT COMPLETELY TRUE. The most active form in terms of antioxidant effect is the tocotrienols (edit to Additionally, α-tocopherol is one of the most biologically active form of vitamin E)

Minor Essential Revision
Check grammar of the under-listed
5. line 26. Unfortunately, it was reported various side effects involving in several organs especially kidney and liver. (edit to Unfortunately, it can produce unwanted side effects in various tissues, including the kidneys and liver)

6. line 40-41. It elicited markedly increased the levels of serum alanine aminotransferase (ALT) (edit to It elicited a marked increase in the levels of serum alanine aminotransferase (ALT))

7. line 44 compared with saline group. (edit to compared to saline group.)

8. line 68 Unfortunately, it was reported various side (edit to Unfortunately, it can produce side effects in various tissues, including the kidneys and liver)

9. line 70... Therefore, this study is emphasized on (edit to Therefore, this study aims to investigate)

10. line 72 ... oxidative stress is believed to cause of (edit to oxidative stress is believed to be involved in)

11. line 83..SOD full meaning before abbreviation (edit to Superoxide dismutase (SOD))

12. line 85 ... Absence of...change to...The absence of.... (edit to The absence of)

13. line 93-94... which is demonstrated the antioxidant ability (edit to which demonstrates the antioxidant ability)

14. line 94. It has been demonstrated as protective agent for prevention of (edit to It has been demonstrated to prevent)
15. line 113. from Division of Animal House *(edit to from the Division of Animal House)*

16. line 115 conducted according to guide for the *(edit to conducted to the Guide for the)*

17. line 123 Wistar rats are divided in five groups. *(edit to Wistar rats were divided into five groups)*

18. line 131. The abdominal cavity of rats is opened, immediately collected liver and then, washed *(edit to After opening the abdominal cavity, the liver was collected and immediately washed)*

19. line 144. was added and mix thoroughly. *(edit to was added and mixed thoroughly)*

20. line 161./172. .... read with a spectrophotometric *(edit to read using the spectrometric)*

21. line 164. Liver was homogenized at 50 mg/ml in cold PBS with 1mM EDTA and centrifuged at 10000 x g, 4 °C for 15 min, and collected Supernatant *(edit to The liver tissue was homogenized to 50 mg/ml in cold PBS with 1mM EDTA, centrifuged at 10000 x g for 15 min at 4 °C and the supernatant was collected)*

22. line 169. and mix thoroughly, *(edited to and mixed thoroughly)*

23. line 296. Histological changes composed comprised of liver congestion, *(edit to Histological changes comprised of liver congestion,)*
24. line 300. resulting in augment of lipid peroxidation (edit to resulting in generation of lipid peroxidation)
25. line 302. antioxidants lead led to the decreased...... (edit to antioxidants led to the decreased)
26. 303 – 304. We suggested that the inefficiency and insufficiency of antioxidant defense system are concerned in some pathological conditions induced by oxidative stress (edit to The inefficiency and the insufficiency of antioxidant defense system were concerned in various pathological conditions induced by oxidative stress.)
27. line 306. Several studies have been focused on (edit to Many studies have focused on)
28. line 312. compound from turmeric which demonstrated (edit to compound from turmeric which has been demonstrated)
29. line 326. vitamin E which acted as potent antioxidant (edit to vitamin E which acts as a powerful antioxidant)
30. line 340-341. However, exact mechanism is still unknown (edit to However, the exact mechanism is still unknown)
31. line 347. This finding indicated that pre-treatment with (edit to These findings indicate that pre-treatment with)