Author's response to reviews

Title: Naja naja atra venom ameliorates pulmonary fibrosis by inhibiting inflammatory response and oxidative stress

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Version: 3
Date: 25 September 2014

Author's response to reviews: see over
Dear editor-in-chief:

We have now revised our manuscript according to reviewers’ suggestions. We hope these revisions have properly addressed reviewers’ questions and concerns. We now send our manuscript to you for consideration of publication in the **BMC Complementary and alternative medicine**. We thank reviewers for their constructive suggestions for improving the quality of our manuscript.

**Reviewer's report**

**Title:** Naja naja atra venom ameliorates pulmonary fibrosis by inhibiting inflammatory response and oxidative stress

**Version:** 2  **Date:** 2 March 2014

**Reviewer:** Masaki Fujita

**Reviewer's report:**

Qin and colleagues have reported an interesting manuscript of new treatment in pulmonary fibrosis. They treated bleomycin- and LPS-induced pulmonary fibrosis model using Naja naja atra venom (NNAV). They found that NNAV attenuated pulmonary fibrosis and the mechanism was inhibited inflammatory response and oxidative stress. Their concept was very unique and the data were notable. However, reviewer concerned the several issues.

**Major Compulsory Revisions**

1. Authors described that Naja naja atra venom (NNAV) was purchased from Yu Jiang, Jiangxi Province, China. Was it certified materials? How did it purified? Did it contain any additives?

**Response**

Naja naja atra venom was produced by a snake farm in Yu Jiang, Jiangxi Province. The quality of snake venom was certified by the Guangxi Institute of Snake Venom (Guangxi, China). It was frozen dried with no purification and did not contain any additives.
2. Authors heated NNAV at 100 C for 10 min. Why did they heat it? Is NNAV heatable? How about using naïve NNAV in this model?

Response

There are several ways to reduce toxicity of snake venom. Zhang (Chinese Pharmacological Bulletin, vol. 17, no. 5, p. 597, 2001) reported that oxidative modification of the NNAV could weaken the toxicity and significantly inhibit the adjuvant-induced edema. We found that a brief heat treatment followed by a slow cool-down increased oral LD$_{50}$ of NNAV from 102 mg/kg to 122 mg/kg. Heat treatment also increased analgesic effect of NNAV (our unpublished observations). Therefore, in this study we applied the heat-treated NNAV to investigate whether it could ameliorate pulmonary fibrosis by inhibiting inflammatory response and oxidative stress.

3. They published several papers using snake toxin and showed anti-inflammatory effect in usually arthritis model. It might contribute to antinociceptive effects of snake toxin. Usually, poisonous snake bite induces severe inflammation. What caused difference between human cases and the present data? Please describe in discussion section.

Response:

The reason why snake bite causes severe inflammation whereas administration of NNAV causes inhibition of inflammation might be due to the way of administration and the dose of NNAV. The therapeutic use of NNAV (microgram range) is much lower than a snake bite (milligram range). The another possible reason is that by oral administration, some components in the venom are destroyed in GI track.

4. Some snake toxin has an anticoagulant property (ProcNatlAcadSci U S A. 2001 Jun 19; 98(13):7230-4). Since anticoagulant therapy might have a benefit for idiopathic pulmonary fibrosis (Chest. 2005 Sep;128(3):1475-82), the role of
NNAV on coagulation system should be discussed.

Response

The effect of NNAV on coagulation system has been discussed in Discussion section.

5. Several mistypes were observed. (eg P9 Effects o)

Response

The mistypes in our manuscript have been corrected and highlighted with red color.

Reviewer's report

Title: Naja naja atra venom ameliorates pulmonary fibrosis by inhibiting inflammatory response and oxidative stress

Version: 2 Date: 4 August 2014

Reviewer: Ali Asghar Hemmati

Reviewer's report:

I have studied the manuscript entitled: Naja naja atra venom ameliorates pulmonary fibrosis by inhibiting inflammatory response and oxidative stress. I wanted to send my comments via the web system however it says the report is overdue. So I send them in this mail as follow:

1- This article is very informative and has valuable contribution to the field of pulmonary fibrosis. An article of outstanding merit and interest in its field.

Response

2- Few typo error was seen in the manuscript which needs to be corrected.

Response

The mistypes in our manuscript have been corrected and highlighted with red color.

3- In the method the time (what day) that blood sample has taken for BGA should be mention.

Response
Arterial blood was collected from abdominal aortic artery after 8 weeks administration of NNAV. This information was now added to the methods section.

4- The discussion of manuscript is mainly the repeat of the results. Discussion should be revised with the purpose of comparison of this work with similar studies. The limitation of this work needs to be declared by authors. Further works for getting better understanding of treatment of pulmonary fibrosis needs to be suggested at the end of manuscript.

Response
According to reviewer’s suggestion, we have now revised the discussion section.

5- Finally my decision about this article is: Accept after minor essential revisions (which the authors can be trusted to make).

Response
Thanks the reviewer. We have now carefully revised our manuscript.

Sincerely yours,

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