Author’s response to reviews

Title: Syzygium jambolanum treatment improves survival in lethal sepsis induced in mice.

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Version: 2 Date: 3 September 2008

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

Title: Syzygium jambolanum treatment improves survival in lethal sepsis induced in mice.

Version: 1 Date: 29 July 2008
Reviewer: Haichao Wang

Reviewer’s report:

Dear Section Editor,

In this manuscript, Maciel et al explored the therapeutic potential of a medicinal herb, Syzygium jambolanum, in an animal model of sepsis induced by cecal ligation and puncture. They showed that a hydroalcoholic extract of Syzygium jambolanum improved animal survival rate, stimulated neutrophil infiltration to site of infection/injury (i.e., peritoneal cavity/cecum), and attenuated systemic accumulation of proinflammatory mediators (such as TNF and nitrite). This is a potentially interesting study with some novel findings. However, there are a few concerns that need to be addressed.

- Major Compulsory Revisions

1). Even in this severe model of sepsis, subcutaneous administration of herbal extract conferred a complete protection against lethal sepsis. This important survival study needs to be repeated at least once in order to increase the sample size to at least N = 20 mice /group. In addition, the animal survival should be monitored for at least two weeks to ensure that this herbal extract did not merely delay the onset of lethality, but rather promote a long-lasting protection against experimental sepsis.

Answer:

In fact the experiments were repeated at least two times and the number of animals/experiment was ten. This information was already described in the material and methods exactly as bellow.

“2.11. Statistical Analysis

Results are expressed as the mean ± standard error of mean (SEM) deviation from 10 animals per group. Statistical evaluation was done by ANOVA test followed by Neuman-Keuls. Mice lifespan was demonstrated using the Kaplan-Meier curve and the log-rank statistical test was applied to compare the curves. Differences were considered significant at P≤0.05 and are represented by an asterisk. All experiments were repeated for at least two times.”
The survival was followed by one month and that mice which had survive until the 5th day remains alive by all the period. This information was now added to the manuscript.

- Minor Essential Revisions
  1). The hydroalcoholic extract contains both alcohol-soluble and water-soluble components. Unlike the water-soluble components, the alcohol-soluble components are probably not soluble in PBS saline. Was there any water-insoluble substance observed after diluting the hydroalcoholic extract with PBS saline?
  **Answer:**
  There was no water-insoluble substance in the extract.

  2). It was not clear why the cecum was punctured eight times, whereas most other investigators only puncture the cecum once or twice to induce sepsis. It may partly explain why most animals lost survival rather quickly (within 1 day) in the present study, as opposed to 2-5 days in most other studies using this model.
  **Answer:**
  The number of punctures in the cecum depends on the needle caliper and on the animals strain. In the beginning of this experiment we had establish the caliper and the number of perforation necessary to induce a lethal sepsis in an early time in our animal house conditions. This procedure is usual to everyone that uses this model, since each animal house shows small differences in the flora found in the mice gut.

  In summary, the authors have described an important finding, which needs to be strengthened with additional experiments as suggested above.
  **Answer:**
  Thank you for your careful appreciation and your kindness suggestions.

**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:**
I declare that I have no competing interests.
Reviewer’s report
Title: Syzygium jambolanum treatment improves survival in lethal sepsis induced in mice.
Version: 1 Date: 9 August 2008
Reviewer: Dong-Keun Song

Reviewer’s report:
Major Compulsory Revisions
Although HCE induces neutrophil accumulation (it is also known to have bactericidal activity in vitro), it did not affect CFU. The author should discuss about the possible reason(s) for this apparent discrepancy.

Answer:
This point was emphasized in the text. In fact, the HCE was used by sc route, so, it had no direct contact with the bacteria. On the other hand, the CFU was performed in the first 12 hours post CLP, what justify the similar amounts of bacteria in the peritoneal cavity. The authors hypothesized that is reasonable to suppose that in this time the bacteria were already not controlled in the initial focus, but the HCE can impair the progression of the infection to a lethal sepses and the disseminated inflammation.

Minor Essential Revisions
seric ---> serum
specie --> species

Answer:
The corrections were accepted. Thank you for the suggestions.

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

Answer:
The language correction was performed.

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: Syzygium jambolanum treatment improves survival in lethal sepsis induced in mice.
Version: 1 Date: 30 July 2008
Reviewer: Lonchin Suguna

Reviewer's report:
Major compulsory revision:
1. The authors have chosen two different concentrations 5mg (low) and 50 mg (high). They may explain the rationale behind in selecting these two extreme concentrations.
   Answer:
   These concentrations were chose based on a previous experiment where we observed a dose dependent recruitment of inflammatory cells by the HCE.

2. The references are haphazardous in the text. The authors may correct this.
   Answer:
   They were corrected.

3. Fig 2 B and C photomicrographs need an embedded scale bar.
   Answer:
   The magnification was cited in the figure legend.

Minor revisions
In page 2, para 2, line 8, the sentence "After 6h it was induced a bacterial infection" could be replaced by after 6h, a bacterial infection was induced specie could be replaced by species wherever it occurs
   Answer:
   ok
The authors have mentioned that the animals had free access to food and acidified water? Why did they use acidified water?
   Answer:
   This was made to avoid any possible water contamination. The control group received the same water.
In page 4, para 2, line 13, each could be replaced by every
   ok
In page 4, para 2, line 16, To could be replaced by For

ok

PBS - is it phosphate buffered saline or phosphate buffer solution. Explain.
The correct is the first one.

Page 4, para 3, line 22, Benjamin et al reference number is not mentioned.
It was mentioned

Allover, no uniformity is maintained in the references in the text.
They were revised .

Page 5, line 9, to could be replaced by for

ok

Page 6, line 3 and 6, previously described could be replaced by previously described methods.

ok

Page 7, line 16, then could be replaced by than

ok

Page 8, last line , and could be replaced by which

ok

Thank you for your careful appreciation and your kindness suggestions.

**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

**Answer:**
The language correction was performed.

**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