Reviewer's report

Title: Protective effects of Zhuyeqing Liquor on the immune function of normal and immunosuppressed mice in vivo

Version: 2  Date: 26 April 2013

Reviewer: Gamal Ramadan

Reviewer's report:

Major Compulsory Revisions

1-In general, the authors use mix of British and American English, which is not acceptable for publication. The authors should use only one type of English language. In many times the same word was written by both British and American English such as "defence and defense".

2-The study did not evaluate the lymphocyte cellularity of spleen and thymus, which is important to determine the effect of ZYQL on the specific immune response more than evaluating the changes in the spleen and thymus coefficients.

3-Results:
-Results are not well described; the statistical analysis in (Table 3) is not matched with the data mentioned under "section 3.3". Either there is a mistake in describing the data in Table 3 or some of the statistical signs are absent in the Table 3. This part of results needs considerable revision.

4-Effect of ZYQL on cytokine secretion levels and lysozyme activity in normal and immunosuppressed mice:

a-The authors suggest that ZYQL dose-dependently enhanced the production of both IL-6 and IFN-# in the serum of normal and immunosuppressed mice. This data is not acceptable. The effect of ZYQL is not a dose-dependent, since cytokines production in the animals received the dose of 200 mg/kg is more than cytokines production in animals received either 400mg/kg or 100 mg/kg of ZYQL. In addition, the value of cytokines did not significantly alter in the normal mice treated with/without ZYQL. Therefore, the sentence should be changed to "ZYQL enhanced the production of both IL-6 and IFN-# in the serum immunosuppressed mice (p<0.01, Table 3)".

b-Please remove this sentence "IL-6 production was augmented in a dose of 200, 400 mg/kg treated with ZYQL in immunosuppressed mice, while that of IFN-# was also highly increased at the dose of 200, 400 mg/kg in immunosuppressed mice."

c-Please replace "Furthermore, ZYQL (200 and 400 mg/kg) increased the activity of LSZ in normal mice" with "Furthermore, ZYQL (100, 200 and 400 mg/kg) increased the activity of LSZ in immunosuppressed mice".
5-Effect of ZYQL on spleen antioxidant enzyme activities in normal and immunosuppressed mice:

a-Please replace "mice treated with ZYQL" with "mice treated with 200 or 400 mg/kg of ZYQL".

b-Please replace "group (p<0.05, p<0.01, Table 4)." with "group (p<0.01, Table 4).".

6-Discussion:

a-Fourth paragraph:

The authors wrote that "The present work exhibits that ZYQL (100, 200 and 400 mg/kg) treated groups significantly improved the cellular LSZ activity of macrophages (p<0.05, p<0.01) in immunosuppressed mice, and ZYQL (200 and 400 mg/kg) treated groups significantly improved LSZ activity (p<0.05, p<0.01) in normal mice. Firstly, according to the material and methods, the LSZ activity was measured in serum and not in macrophages. Secondary, Table 3 demonstrated that there were no significant changes in LSZ activity in ZYQL only treated animals compared with the healthy control animals. Therefore, this sentence is not in agreement with the results in Table 3.

b-Sixth paragraph:

According to the data in Table 4, please replace the phrase "in both normal and immunosuppressed mice except the group of 100 mg/kg ZYQL (p<0.05, p<0.01)," with the phrase "in both normal and immunosuppressed mice (p<0.01), except in the group of 100 mg/kg ZYQL, ".

c-Seventh paragraph:

According to the data in Table 4, please replace the phrase "the level of CAT both in normal and immunosuppressed mice (p<0.05, p<0.01)." with the phrase "the level of CAT both in normal and immunosuppressed mice (p<0.01), except in the group treated with 100 mg/kg ZYQL only.".

7-References:

a-Three references are not found in the reference list at the end of the manuscript, while they are cited in the text:

-Beauchamp and Fridovich (1971).

b-Please make the scientific name of pants and animals italics such as:
-Glycyrrhiza uralensis.
-Angelica sinensis.
-Angelica sinensis.
-Candida albicans.
-Astragalus membranaceus.
8-Tables:
-Please remove this sentence "#Significant difference at p<0.05 levels compared with normal control group." from Tables 3 and 4.

Minor Essential Revisions
1-Author list:
-Hang-Yu Wang b*: I think the star should be removed.

2-Abstract:
a-by intraperitoneal injection: Please add "on days 4, 8 and 12" after the word "injection".
b-Please replace "uprate" with "upgrades".
c-serum lysozyme, promoted: Please add "as well as" instead of "the comma" after the word lysozyme.
d-Please correct the typing mistake in the word "founctional".

3-Introduction:
a-Second paragraph:
I-Please replace "In the study" with "In the present study".
II-Please replace "a few medical herbs such as" with "the chemical properties and biological activities of".
III-Please remove the phrase "in their chemical properties and biological activities" after the word "studied".
IV-Please replace "above on" with "on the above".

b-Third paragraph:
I-Please replace "Cy effects" with "Cy affects".
II-Please replace "and antioxidant defences and" with ", antioxidant defences, and".

3-Fourth paragraph:
I-Please replace "in in vivo" with "in vivo".
II-Please replace "Cyclophosphamide (Cy, 100 mg/kg)" with "Cy (100 mg/kg)".
III-Please replace "treated with Cyclophosphamide" with "treated with Cy".

4-Materials and methods:
a-Experimental animal and design:
I-Second paragraph:
-Please replace all "wherein the day of" with "wherein the days".
-Please add "; cyclophosphamide (Endoxan®) was purchased from the affiliated hospital of Shihezi University, Xinjiang, China" after "100 mg/kg Cy".
Please replace "(100, 200 and 400 mg/kg)" with "(100, 200 and 400 mg/kg, respectively)".

II-Third paragraph:
Please add °C after "-80".

III-Fourth paragraph:
Please replace "Another Eighty BALB/c mice used" with "Other eighty BALB/c mice were used".
-Please replace "method samed" with "design was the same".

b-Biochemical analysis:
I-Please add ", according to the manufacturer's protocol. The observation absorbance of the reaction was read at 450 nm" after "Sigma-Aldrich Chemicals Co., USA".

II-Please remove this paragraph "Cyclophosphamide (Endoxan®) was purchased from the affiliated hospital of Shihezi University (Xinjiang, China). Ink staining was purchased from Beijing Xizhong chemical plant (Beijing, China)."

c- Carbon clearance methods:
I-Please replace "All the mice" with "all the mice used to detect the phagocytic function of mononuclear phagocytic system with carbon clear up methods".

II-Please replace "inject Ink diluents (1:5 dilute) 0.1ml/10g by caudal vein" with "injected Ink diluents (1:5 dilution, 0.1ml/10g) in the caudal vein".

III-Please replace "30" with "Ink staining was purchased from Beijing Xizhong chemical plant (Beijing, China). Thirty".

IV-Please replace "content with" with "contains".

d-Enzyme linked immunosorbent assay for IL-6, IFN-# and Lysozyme activity:
I-Please remove "2.5 Enzyme linked immunosorbent assay for IL-6, IFN-# and Lysozyme activity".

II-Please remove this paragraph "Serum IL-6, IFN-# and Lysozyme activity were measured by the enzyme-linked immunosorbent assay method using the kit obtained from Sigma-Aldrich Chemicals Co., USA, according to the manufacturer's protocol. The observation absorbance of the reaction was read at 450 nm.".

e.Determination of antioxidant enzymes activity:
I-Please remove "2.6 Determination of antioxidant enzymes activity".

II-Please remove "in absorbance".

III-Please remove "SOD activity of liver homogenate was determined from a standard curve of the percentage inhibition of NBT reduction with standard SOD activity".

IV-Please replace "SOD activity was assayed" with "SOD activity in spleen homogenate was assayed".
V-Please transfer all the paragraphs (in section 2.6) started from "SOD activity in spleen homogenate was assayed .................. till CAT activity was expressed as U/mg protein" behind "from Jiancheng Institute of Biotechnology (Nanjing, China)." (in section 2.3.).

f-Statistical analysis:
I-Please replace "2.7" with "2.5".
II-Please replace "Data" with "data".

5-Results:
a-Effect of ZYQL on organ coefficient in normal and immunosuppressed mice:
I-Please replace "Cy-treatment (p<0.05, p<0.01, Table 1)" with "Cy-treatment (p<0.05 and p<0.01, respectively, Table 1)".
II-Please replace "enhanced" with "alleviated".
III-Please add "induced by Cy" after "coefficient".
IV-Please remove "individually".
V-Please remove "unfortunately".
VI-Please replace "of ZYQL. These results are presented in Table 1." with "of ZYQL (Table 1).".

b-Effect of ZYQL on Phagocytotic function of mononuclear phagocytic system
I-First Paragraph:
-Please replace "regard" with "regarded".
II-Second Paragraph:
-Please add "for" before "fourteen".
-Please replace "(p<0.05, p<0.01, Table 2)." with "(p<0.05-0.01, Table 2).".
-Please replace "was not exhibit the enhancement" with "was no enhancement in the".

c-Effect of ZYQL on Cytokine secretion levels and Lysozyme activity in normal and immunosuppressed mice:
I-Please remove this part "To assess the effects of ZYQL on the secretion of cytokines and Lysozyme activity, normal and immunosuppressed mice treated with various concentrations of ZYQL up to 14 d. The levels of IL-6, IFN-# and Lysozyme activity were analyzed by ELISA Method.".
II-Please replace "Cy group (p<0.05, p<0.01, Table 3)." with Cy group (p<0.05-0.01, Table 3).

d-Effect of ZYQL on spleen antioxidant enzyme activities in normal and immunosuppressed mice:
I-Please replace "immunosuppress" with "immunosuppression".
II-Please replace "mice(p<0.05, p<0.01, Table )," with "mice (p<0.05-0.01, Table 4),".
6-Discussion:
a-First and Second paragraphs:
I-Please replace "Cyclophosphamide (Cy)," with "Cy,  ".
II-Please add "Comma" after "Therefore" and after "such as autoimmune diseases".
III-Please replace "treatment" with "treat" and "precaution of" with "protect from".
IV-Please remove the word "effects" after the word "difficult".
V-The two paragraphs started from "Cyclophosphamide (Cy),……………… till Western medicine" should not be in the discussion section and may be added to the introduction after making it shorter.
b-Third paragraph:
I-Please correct the typing mistake in "displaied".
II-Please replace "ZYQL educe the protective effects may be through the non-specifical immunity system." with "ZYQL may enhance the non-specific immunity by increasing the phagocytic activity of macrophages.".
c-Fifth paragraph:
I-Please replace "interfere on" with "interferon".
II-According to the data in Table 3, please replace "while in the normal mice there was not a significant difference for the cytokines IFN-#." with "while it did not significantly change these cytokines in normal mice.".
d-Eighth paragraph:
I-Please add "s" for the word "exhibit".
II-Please replace "immunosupressed mice (p<0.05, p<0.01)." with "immunosuppressed mice (p<0.05-0.01), except in the group treated with 100 mg/kg ZYQL only.".
e-Ninth paragraph:
I-Please remove "100," from "(100, 200 and 400 mg/kg)".
II-Please replace "(p<0.05, p<0.01)" with "(p<0.05 and p<0.01, respectively)".
III-Please replace "difference in the normal mice" with "difference in thymus and spleen indices in the normal mice treated with ZYQL".
f-Conclusion:
I-Please replace "above, on the one hand, we can come to the conclusion" with "above, we can conclude".
II-Please replace "that it had" with "that ZYQL had".
III-Please replace "the normal mice and Cyclophosphamide-induced" with "the Cy-induced".
7-Please replace "glutathion" with "glutathione" throughout the manuscript.
8-Please replace all "In the fifteenth days," with "On the fifteenth days," throughout the manuscript.

9-Please correct the typing mistake in the word "immunosuppressed" throughout the manuscript.

10-Authors' contributions
- The contributions of the following two authors (Guo-Yu Li and Jian Huang) are not cited.

11-Tables
a-Please replace "s" in Tables 1-4 with "SD".
b-Please replace "Phagocytotic" in Table 2 with "phagocytotic".
c-Please replace "Cytokine" in Table 3 with "cytokine" and "Lysozyme" with "lysozyme".
d-Please replace "prot." in Table 4 with "protein".

Discretionary Revisions:
- It is of great value if the authors investigate the effects of the ZYQL drinking on liver functions. Investigation the effect of ZYQL drinking on liver weight is not enough to detect or exclude any toxicity for the Liquor drinking on the liver.

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.