Author’s response to reviews

Title: The immunomodulatory activities of licorice polysaccharides (Glycyrrhiza uralensis Fisch.) in CT 26 tumor-bearing Mice

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Response to Reviewer’s comments

Reviewer 1

- Background: Line 58, I have added …one of the leading…..and in 2013, the second after cardiovascular diseases

- Chemical characterisation of plant material used.

Sample material used was provided by TUTCM pharmaceutical company.

We have included a brief preparation of the extracts in the section of materials and methods, heading, Preparation of Licorice polysaccharides

- In analysis by FACS Calibur should be added more information, gating parameters? which types of antibodies were used?

Procedure expanded to include more information including the antibodies used. Section, immune cell population, line 173-186.

- Using S.E.M is not proper. SD should be use

Thank you. We have changed and used SD as advice
- Statistical analysis should be performed more professional. How normality was verify?
Yes statistics were done professionally and SPSS software was used for analyses.
Normality was tested and the data found to be normally distributed. This has also been added to the statistical analysis section line 199-200

- Why Authors use one way ANOVA? (equality of variance?)
Thank you. This was used because of our single factor, Licorice extract

- In table and figure descriptions n number should be given
On tables in the manuscript, we have also added descriptions and numbers to all tables.

- In table 4 concentration should be added
Thank you. The concentration (pg/ml) has been added to the table.

- IL-7 in work has minimum 3 different types of description
Thank you for highlighting this, we presume that you are referring to the different description of IL-7 it appears in the manuscript. We have harmonised.

Reviewer 2

- The manuscript has some typos and grammatical errors which need to be rectified in the text of the entire manuscript.
Thank you for keenly going through our manuscript. We have made quite a number of grammatical changes and typo correction, in the entire text, deleted some paragraphs and sentences in the entire manuscript

- Please also correct the Title of the manuscript.
Thank. We have revised the title to….The immunomodulatory activities of licorice polysaccharides (Glycyrrhiza uralensis Fisch.) in CT 26 tumor-bearing Mice

- Define all abbreviations in the text when used for the first time.

Thank you. We have defined the abbreviations, and also given the list of abbreviations at the end of the manuscript in the section of …List of abbreviations

- What is BRMs? "

BRMs stands for Biological response modifiers, we have defined this in section of conclusion, line 482.

- In vitro and In vivo" should be written in italics.

Thank you. We have italised the two words

- Licorice extract is not active as it increases the spleen size more than 4 times after treatment as compared to normal control (Table-1). Authors should perform the blood parameter for red blood cells, white blood cells, and platelets counts.

Thank you for the concern. Yes blood parameters are also important in showing the activity of the extract, but also, the immune activity can be checked by looking at the size of the spleen, thymus which are an immune organs. We have added a reference to support our conclusion on spleen and thymus size and indices. Shenshen Zhang, Shaoping Nie, Danfei Huang, Wenjuan Li, Mingyong Xie. Immunomodulatory effect of Ganoderma atrum polysaccharide on CT26 tumor-bearing mice

- Authors need to present all data in the form of graphs instead of tables.

Thank you for the suggestion. We believe tables could present our results accurately. We have also used figures to represent the results for cytokine levels i.e figure 2.

- Flow cytometry data (table-3) can be presented as Scatter Graphs along with density plots.
Thank you. We choose tables to show clear figures. We have also added a figure for dot plots.

- Statistical analysis should be verified. It is not clear how the measurements are compared between groups.

A statement has been added under statistical analyses section, line 244-246 to describe how comparison was done between the treatment groups.

- Please, revise carefully the statistic symbols and the level of significance.

Thank you. This has been harmonised on all tables and figures.

- 500mg/kg dose was used in the study, which is very high. -How was the dose of licorice extract in mice chosen- were there preliminary experiments to justify this?

Thank you. The dose used was preferred after in vitro studies with the extracts. A reference for earlier in vitro work that guided this has been added. Reference 10.

- How were the fractions A, B & C prepared?

Thank you. The preparation of these fractions have been added to the section of materials and methods, title, Preparation of licorice polysaccharides.

- How were T-lymphocytes isolated from the peripheral blood. Please add this information.

- Is the dose of licorice extract used, similar to those physiologically observed when human consume natural products?

It is not the same. The concentration used was arrived at after our earlier in vitro test.

- The important mechanism of this paper is that licorice extract activates T-cells by the upregulation of IL-7 in the mice; however, how this happens is not addressed experimentally and probably should be.
Thank you. The mechanism is by immunomodulatory activities, among them immune organ index, T lymphocyte activation and cytokine production. IL-7 is one of the cytokine evaluated. But inference is linked to other studies that link activation of T cells by IL-7.

- Results are not presented accurately in the manuscript. Please revise it carefully.

Thank you. We have revised the entire result section

- What is growth (weight) in table-2?

We have reorganised the table 2 and changed from growth to weight.

- Table-2 is just the repetition of table-1.

Thank you. Noted and changed to avoid repetition

- Please also mention units in the entire manuscript, example; page-7, line 194 25.03±1.52 should be written as 25.03±1.52 gm. Same goes for all of the other values used in the study.

Thank you for noting this mistake. We have made the changes. We have include the symbols of measurement i.e results section line 255, table 1, line 299-300 we have added grams (g) and percentage (%)

- The introduction can be shortened by using only relevant references.

Thank you for the direction. We have shortened the introduction and deleted several sentences i.e line 92-95, 100-104, 107-109, 123-125, 129-138, 140-146 and 148-151

- Discussion can be improved. Some sentences from the Results and Discussion sections are required to be reframed to make them more meaningful.

Thank you. We have also improved the discussion by adding and removing some sections i.e line 331-332, 339-348, 357-393, 427-437, 462-478