Author’s response to reviews

Title: Water-separated part of Chloranthus serratus alleviate lipopolysaccharide-induced RAW264.7 cells injury

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Reviewer reports:

Sawsan Zaitone (Reviewer 1): Article entitled "Water-separated part of Chloranthus serratus alleviate lipopolysaccharide-induced RAW264.7 cells injury" by Sun et al. investigated the antiinflammatory effect of Water-separated part of Chloranthus serratus al. against LPS induced cell injury. They used ELISA, immunohistochemical and Western blot assays for investigating this effect.

In general the study provides novel data for this water fraction of C. serrata and data are supported by the conclusion, however, some major points need to be revised.

Title: need to indicate the mechanism for the anti-inflammatory activity focusing on AMPk Nrf2-OH1 pathways

Answer:

Title of the manuscript is modified as follows: water-separated part of Chloranthus serratus alleviate lipopolysaccharide-induced RAW264.7 cells injury mainly by regulating the MAPK and Nrf2/HO-1 inflammatory pathways, which indicates the mechanisms of anti-inflammatory activity.
Methods:

If there is a rational for the selected dosed of the water extract

Answer:

The low, medium and high doses were optimized by the following experiments. Firstly, CCK-8 method was used to detect the cell viability and preferably select the concentration range. Then, the cells were treated with different concentrations of water-separated part of Chloranthus serratus and 1 µg/mL LPS for 24 h. The supernatant was collected to determine NO concentration. The protein was extracted and the content change of HO-1 protein was detected by Western blotting to further optimize the suitable gradient concentrations. Finally, the final concentrations of the experiment were selected according to the both cell viability and anti-inflammatory effect after administration.

Method of ROS is not complete, kindly give full method and details of kits for all procedures

Answer:

A complete method for ROS determination has been supplemented in the text (P8).

no information about the post-hoc analysis after ANOVA

Answer:

LSD was performed for the post hoc test (P8).

Did authors test the normality of distribution of data before applying ANOVA, if data are not in normal distribution, non-parametric ANOVA should be applied?

Answer:

Before analyzing the data, we first tested the normality of the data distribution. Results showed that all the data were normally distributed.

Results: western blots should be demonstrated clearly, some bands arranged vert sticky to each other and difficult to interpret

Answer:

Fig. 5 Effects of CSSPW on the expression of Nrf2/HO-1 pathway in LPS-induced RAW264.7 cells (P13). The display of the bands has been modified.
Fig. 6 Effects of CSSPW on the expression of MAPK pathway in LPS-induced RAW264.7 cells (P14). The display of the bands has been modified.

Naoko Ikuta, Ph.D. (Reviewer 2): In this study, authors evaluated anti-inflammatory effects of CSSPW dose-dependently, and they supplied its potential mechanisms, which are useful information for pharmaceutical applications. Their study is technically designed, the results are novel and the manuscript is well structured and easy to read and follow.

In conclusion, considering the above matters, I retain that this manuscript could be accepted for publication in BCAM, after the following revision.

Extensive editing of English language and style should be required, too.

Answer:

We have taken American Journal Experts (http://bit.ly/AJE_BS) to help with English and modify the language of the manuscript, hoping to further improve the level of the manuscript.

Major revision:

1) Authors explained the preparation of CSSPW in detail, but they did not show its contents. Please show the contents of CSSPW if they were evaluated it. It is important what compounds give the anti-inflammatory effect.

Answer:

The root of Chloranthus serratus contained dihydropyrocurzerenone, pyrocurzerenone, shizukanolide E, F, neoacolamone, 7-α-hydroxyneo-acolamone, acoragermacrone, acolamone, zederone, isofuranodiene, furanodiene, chloranthalactone C, shizukanolide C, etc.

The content of the CSSPW in the roots of C. serratus was 1.94%. CSSPW had absorption peaks at 367 nm, 359 nm, 323 nm, 301 nm, 280 nm and 259 nm (P17).

Up to now, it is known that the terpenoids with relatively large polarity play an anti-inflammatory role. However, the specific anti-inflammatory effects of which terpenoids remain to be studied. Due to a lack of in-depth research, it is still impossible to buy the standard. Therefore, the content of specific effective components can’t be determined at present. The purpose of this study is to lay the foundation for the separation of its anti-inflammatory monomer components, and gradually separate the anti-inflammatory monomer components in the future.

Minor revision:
1) Page 10, in Figure 2: Please add the scale bar onto the individual photo.

Answer:

The scale bar has been added (P10, Fig. 2).

2) Page 8. In Line34: Please explain the calculation of ROS generation ratio. To see page 16, in figure 9, ROS expression percentage is low, the control showed less than 1 % and LPS showed around 4 %. Relative Fluorescence Units (RFU) would be better.

Answer:

ROS generation ratio was obtained by ImageJ Software. Relative Fluorescence Units (RFU) had been adopted (P16, Fig. 9).

Dear editor, we greatly appreciate your help. We have further explained and revised the arisen problems, and hope that the revised manuscript is more suitable for publication.

Best wishes,

Shuping Sun, Yunyan Du, Chuanliu Yin, etc.