Author's response to reviews

Title: Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis

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Author's response to reviews: see over
Dear editor,

According to the reviewers’ comments, we revised the manuscript as follows.

Comment 1:

1 In the Instruction section, please explain why corilagin was chosen in this study.

Response: In introduction, we added “As nowadays there are no specific remedies for cholestasis, and Corilagin can alleviate the impairment caused by inflammation and oxidation, we chose Corilagin to…….” in order to explain the reason for choosing Corilagin.

2. In this study, 90 animals were divided into 5 groups, which indicated that each group had less than 20 animals. The results would be more convincing with more animals in each group.

Response: In each group there were 18 rats and each 6 rats for one time-point. Although the number of rats was limited, there were 30 rats for sacrifice in each day. We would extend our research group and enlarge the work scale in our further experiments.

3. Please clarify each group in detail in the Results section.

Response: We added the result of each group in related section.

4. It is suggested that all author go over the whole manuscript to correct English writings as a group.

Response: We carefully checked the English writing in the manuscript.

Comment 2

Major Compulsory Revisions

1 The purity of the prepared Corilagin with 62% seems quite low. Can the authors exclude a cooperative effect of any substance in the remaining impurities of 38%?

Response: Although Corilagin can be chemically synthesized, the production is too little and too expensive to be enough for animal experiment. So we chose to extract Corilagin from plant. Actually there has not been special method to exclude a cooperative effect of other substance in the Corilagin extraction so far. Sometimes the ingredients in medicinal plant are too complicated to be defined which one is ineffective. Thereby in some researches on medicinal plant it permits the unpurified
2 The entire manuscript is full of mistakes regarding use of tenses, prepositions and word choice. At some points, the standards for scientific writing are missing. The authors should look for the help of a native English speaker to improve the quality of their text. This reviewer will only suggest corrections for a few errors (from Material and Methods, p. 7-10):

a. Chemicals and reagents
All chemicals were purchased from … (like PBS and other basic stuff) unless indicated otherwise. Affinity-purified rabbit anti-rat NF-κB p65 was received from Santa Cruz Biotechnology (Santa Cruz, CA, USA)…
Biotin-conjugated goat anti rabbit IgG and streptavidin-horseradish peroxidase (HRP) conjugate are missing. Assay kits were moved to another paragraph, source of ANIT (write out here and not until discussion) and UDCA can also be moved to paragraph “Model and control establishment”.

b. Immunohistochemistry assay
SP (probably Streptavidin peroxidase, write out when used first time) immunohistochemical assay was employed to detect expression of the nuclear translocation of NF-κB. The slides of hepatic tissue were soaked in 3% H2O2-methanol solution for 20 min in order to block endogenous peroxidase. In the next step 1% Triton X-100 was added at 37°C for 5min, followed by washing with PBS. After incubation with normal goat serum at room temperature for 20 min, rabbit anti-rat NF-κB p65 IgG antibody (dilution; if used undiluted: volume) was added dropwise and the slides were stored at 4°C overnight. The next day slides were washed with PBS and incubated with biotin-conjugated goat anti rabbit IgG for 30 min at 37°C. After another washing with PBS streptavidin-HRP was added and incubated for 30 min at 37°C. The slides were thoroughly washed with PBS 3 times for 5 min and stained with 3,3’-diaminobenzidine. Following normal dehydration, lucidification and mounting the slides were analysed under microscope model (manufacturer, country) as specified in our previous studies [18, 19, 20, 21] and digital images were captured with camera model (manufacturer, country).

c. Analysis of NO and markers for oxidative stress
Myeloperoxidase (MPO), malondialdehyde (MDA), superoxide dismutase (SOD), and nitric oxide (NO) were quantified by the respective assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the instructions of the manufacturer. The procedures were also described in our previous studies [22, 23].

d. Purity determination of Corilagin
Purity of Corilagin was determined by HPLC. The procedure abided by our previous study [24, 25]. Briefly, a Hanbon-Kromasil 5μm C18 column was used at 30°C with the wavelength of 268nm for detection. The mobile phase was composed of 0.5% phosphoric acid and methyl cyanide with a ratio of 76:24 and the injection volume was 10μl. Corilagin standard substance (purity>99%) was offered by China National Institute for the Control of Pharmaceutical and Biological Products. The purity of Corilagin was calculated to 62.14%. (Description of the method belongs here and not
in Results section.)

Response: We corrected the writing above accordingly and checked and revised other expression in the manuscript carefully.

3 Figure legends 1-4: Omit liver protective effect at the beginning, this is yet an interpretation of the data and belongs to the discussion. Write just what is shown in the figure.

Response: We omitted the liver protective effect at the beginning from figure legends 1-4 and changed the number of all figures.

4 A more precise title would be: “Anti-inflammatory and anti-oxidative effects of Corilagin in a rat model of acute cholestasis”

Response: We change the title of manuscript accordingly.

Minor Essential Revisions

1) Figure legend 3: Numbers 4A etc. are wrong.

Response: We corrected the number of related figure legends.

2) Page 6: TBHP should be written out.

Response: We altered the full name of TBHP.

Response to the editor

We added the 'Competing interests' and 'Authors' contributions' section according to your request.

If you have further requirements, please let us know. Thank you very much.

Lei Zhao