

Response to Reviewers:

Reviewer #1: In the manuscript 'Tamarix Chinensis Lour Inhibits Chronic Ethanol-Induced Liver Injury via the NLRP3-caspase-1-IL-1 β Pathway in Mice', the researchers fed C57BL/6J male mice with Lieber-DeCarli lipid diet containing alcohol to model ALD mice and then treated them with different doses of TCL. Indexes for hepatotoxic and inflammasome and cytokines were detected. This research was focused on the hepatoprotective function of TCL extract treatment in ALD and put forward a point that inhibiting NLRP3-caspase-1-IL-1 β signaling pathway may be involved in this protective process. This interesting study was designed well with a rich supportive data and reliable statistical analysis, however, there are some questions for it:

1. The protective role of TCL has been proved in liver disease, what mechanism does it involve? Is it associated with NLRP3? Are there any other researches focused on NLRP3 in ALD? If so, please detail it and elaborate on the innovation of this study.

A: Alcohol and its metabolites can cause oxidative stress in the body and lead to liver chemical damage. As shown in Table 1, the study on the protective effect of Tamarix extract on liver injury is mainly focused on improving the antioxidation of the liver. We supplemented the research on NLRP3 in ALD in the second half of the first paragraph of the introduction, but there is no literature reports on whether CTL affecting on NLRP3 in liver disease.

2. If other mechanisms are involved in the function of TCL in liver protection, how can you explain the successive relationship between inhibition of NLRP3 and downstream cytokines in liver protection? How do you confirm that the decrease of the inflammasome is caused by TCL and then protect the liver function? The successive relationship between NLRP3 and ROS pathways needs to be explained as well.

A: The relationship between NLRP3 and downstream cytokines was described in the first paragraph of "Introduction" of this paper. The main mechanisms of TCL are to protect liver through antioxidation. The results have verified that TCL can regulate the levels of NLRP3 and its downstream cytokines at the gene and

protein levels respectively.

In the second paragraph of “Discussion”, we supplemented the relevant evidence description of the relationship between ROS and NLRP3.

3. According to your results, GSH has a similar effect compared with a high dose of TCL in almost all the indexes, did they share a similar mechanism? If not, the successive relationship mentioned above should be re-explained.

A: GSH is the abbreviation of glutathione. Its main physiological functions are scavenging free radicals, anti-oxidation and anti-aging. This study showed that GSH also had a significant regulatory effect on NLRP3 inflammasomes, and the mechanisms are similar to TCL in terms of available data. The relevant content was added in “Animals and treatment” section and the third paragraph of “Discussion”.

Reviewer #2: The author’s purpose of the investigation is very interesting, also for medical and/or scientists from related research fields. I would recommend the suggestions described below:

- 1) The title should be short and concise. According to recent studies that would favor future citations to the paper. What is really new in the paper?

A: In our manuscript, the protective effect of TCL extract on alcoholic liver injury was observed. The results showed that TCL could alleviate alcoholic liver injury in mice by inhibiting the activation of NLRP3-caspase-1-IL-1 β pathway. The title of the paper "Tamarix Chinensis Lour Inhibits Chronic Ethanol-Induced Liver Injury via the NLRP3-caspase-1-IL-1 β Pathway in Mice" summarizes the main contents of the study. If the title was further simplified, it will not faithfully reflect the full picture of this study and its core content.

- 2) Abstract should be also quantitative as possible for rapid comparison with similar studies. Avoid imprecise terms such significant inhibition...but how much? –....” reduction of hepatic tissue ROS and MDA levels, with a significant increase of SOD” : but how much 100%?; 2-fold?. On the other hand, if the effect is not statistically significant no need to mention it because it could be only a tendency

not a fact.

A: We adopted reviewer's advice, revised the abstract, added data description in "Results", and deleted uncertain descriptions such as much, as shown in "Abstract".

3) The paper includes 7 references from the last 4 years (about 25% of the total). However, by increasing this percentage with recent papers from 2019 and 2018, for similar studies with other compounds which would turn the paper real and timely.

A: According to the suggestion of reviewer, we adjusted and supplemented the References in the citation and increased the number of references in the past five years, such as references of No.2 (2019), No. 5 (2015), No. 20 (2019), No. 21 (2018), No. 31 (2106) and No. 34 (2015).

4) Introduction should be less general and focuses in the main message of the paper. At the end of the intro, it is also not clear what is the main message and relevant points of the paper that should be emphasize at this stage.

A: We agree with reviewer's proposal to modify the introduction. 1) The second half of the first paragraph, "Inflammasome activation is a 2-step process, in which lipopolysaccharide serves as the first signal that upregulates NLRP3 inflammasome components and pro-IL-1 β expression via NF- κ B-dependent pathways. Then, a second signal activates NLRP3, which is usually one of the damage-associated molecular patterns (DAMPs) such as ROS, ATP, or uric acid crystals. NLRP3 recruits ASC and pro-caspase-1, and the NLRP3 inflammasome is assembled", changed to "When NLRP3 inflammasome activated, the inactive pro-caspase-1 is cleaved to form active caspase-1". 2) The last paragraph of Introduction was modified and added.

5) The authors referred that *Tamarix chinensis* Lour (TCL, Tamaricaceae) is a shrub that usually grows in arid or semiarid desert areas and saline-alkali fields. It is a traditional Chinese herbal medicine with hepatoprotective, antioxidant, antibacterial, and antitumor activities. I would like to make the following suggestion: At the

introduction a figure or scheme with the chronology or a timeline of the applications of TCL as anticancer, antimicrobial and other major events in the field would be interesting and useful for a better understanding of the paper. Moreover, this timeline will also reflect the understanding of the authors about the major milestones for TCL in medicine and in particular for hepatic protection. This personal view timeline will be interesting and also pedagogical for chemists, biologists, researchers and professors in the field as well as also for medical doctors and/or scientists. It could be a specific timeline only for TCL compounds in medicine. Many combinations are possible according to author' s desire as a take home message within this project.

A: We agree with reviewer's recommendation to summarize the pharmacological effects of Tamarix extracts distributed all over the world. As shown in the attached table (Table 1), there are few pharmacological studies on TCL extracts distributed in the desert areas of China.

However, whether the table should be listed in the paper needs to be negotiated and confirmed with the editors. We tend not to publish the table in the article.

6) A figure for the shrub TCL could also favoured the paper.

A: This is our collection of Tamarix canopy growing in the sandy soil along the coast of the Bohai Sea in China in spring. It was inserted in "MATERIALS AND METHODS" as Figure 1.



7) The authors should explain why mice in the TCL-treated group (200 mg/kg) were used. Is this amount similar to others antioxidants used?

Comparison is a step further in science.

A: The doses of 100 mg/kg and 200 mg/kg TCL extracts used in this paper were based on the published research papers [Urfi et al.2018] on Tamarix extract for antioxidation and protection against chemical liver injury. At the same time, we also made reference to the dosage of TCL decoction in Chinese traditional medicine, and confirmed the corresponding dose of mice according to the dose of human administration. The relevant contents and reference were supplemented in “Animal and treatment” of the manuscript.

8) Globally, the results are not properly described. The authors should first describe in a quantitative manner the data before jump to conclusions. Avoid imprecise and/or qualitative terms such as reduced.. increase....

A: As mentioned by reviewer, we described in detail the data in the presentation of the Results and in the results chart description, especially the comparison between the experimental group of TCL treatment and the alcohol model control group used specific data. Please refer to the revised Results.

9) The figures should be clearly globally improved, as possible, once WJG deserves high quality figures and with rigor would avoid lacking of interest for the data. Legends should be also as complete as possible. Scale bars are missing in the Fig 2 legend?

A: We agree with reviewer, this research project adopted the method of inter-group comparison, so we supplemented the relevant data in manuscript, with data to reflect the protective effect of CTL on liver injury as much as possible. At the same time, according to reviewer's suggestion, we replaced a clearer picture (revised Figure 3) of the pathological tissue.

10) A scheme highlighting to putative mechanisms of action for TCL would favor the message within the study.

A: We agree with reviewer, it was found in the initial study of the project that TCL has a certain inhibitory effect on liver oxidative stress induced by alcohol. Because ROS plays a role in the activation of NLRP3 inflammasomes, we

speculate that TCL may play a role in regulating the signal pathway of NLRP3 inflammasomes, so we carried out this study and achieved the expected results.

11) Discussion should be more assertive and concise and eventually be divided in sections with titles highlighting the major results.

A: According to the suggestion of reviewer, we adjusted and added the discussion contents. Combined with the results, we focused on discussing the role of TCL from pathological changes, oxidative damage, changes of NLRP3 inflammasomes and NKT cells, and added small titles according to the content.

12) A conclusion section, paper with partial conclusions first and then global conclusions would also favor the take home message of the paper.

A: According to the suggestion of reviewer, we adjusted the conclusion and delete some unnecessary descriptions.

Table 1. The pharmacological research methods, pharmacological activities and references of Tamarix species in the world.

| Pharmacological activity | Dosage or concentration | Model/ method | Mechanism | References |
|--------------------------|-------------------------|---|--|---|
| Antioxidant | 500µg/ml | DPPH radical, superoxide anion, and iron chelating activity in vitro. | Highest antioxidant activity. | <i>Drug Discov Ther, 2008</i> |
| | 100mg/kg | ALX-induced diabetes in rats in vivo. | GSH increased | <i>Pharm Biol, 2011</i> |
| | | H ₂ O ₂ -induced oxidative stress in human IEC-16 cells in vitro. | Cell viability increased, LPO reduced, and JNK/MAPK pathways were regulated. | <i>Biomed Pharmacother, 2017</i> |
| Hepatoprotective | 12.5% | CCl ₄ - and CalN-induced model in vitro. | ALT decreased | <i>J Ethnopharmacol, 1987</i> |
| | 50mg/kg | Thioacetamid-induced model in rats; | Reduced the levels of AST, ALT, GST, XO, LPO, LDH, GGT and H ₂ O ₂ , but increased GSH and SOD activity. | <i>Life Sci, 2006b</i> |
| | 100mg/kg | | Returned the altered levels of SGOT, SGPT, and ALP. | <i>Pharm Bio, 2011</i> |
| | 100mg/kg | CCl ₄ -induced model in rats. | Reduced the levels of AST, ALT, TNF-α, NF-κB, COX-2, α-SMA, but increased GSH. | <i>Molecules, 2018</i> |
| | 100,200mg/kg | CCl ₄ -induced model in rats. | Reduced the levels of SGPT, SGOT, ALP, SBL and LDH. | <i>J Diet Suppl, 2018</i> |
| Anti-inflammatory | | Rifampicin plus isoniazid-induced model in rats | | |
| | 100mg/kg | Carrageenan or yeast-induced model in mice. | Antipyretic effects | <i>Pakistan Pharmaceut Sci, 2014</i> |
| | 100µg/ml | LPS-activated microglia cells in vitro. | TNF-α, IL-1β, IL-6, iNOS and NO levels were reduced. | <i>Phytomedicine, 2018</i> |
| Anticancer | 100µg/ml | Human gastric cancer cells in vitro. | Inhibited IL-8 secretion of cancer cells; | <i>J Ethnopharmacol, 2012</i> |
| | 100µg/ml | Human breast and colon cancer cells in vitro. | Inhibited cell proliferation, and angiogenesis. | <i>BMC Complement Altern Med, 2014</i> |
| | 250µg/ml | Rat brain tumor and human cervix carcinoma cells in vitro. | Inhibited cell proliferation. | <i>Acta Scientifica Naturalis, 2018</i> |
| Antimicrobial | 500µg/ml | Agar dilution method against five bacteria in vitro. | MICs: 62.5-250µg/ml for different isolated polyphenols. | <i>Nat Prod Res, 2017</i> |
| | 1000µg/ml | Disc diffusion method in vitro. | MICs: 10-100µg/ml, Inhibition zones: 2-16mm. | <i>Int J Food Prop, 2018</i> |