

Dear Mr. Ma,

Thanks for your consideration on our paper titled “Naturally derived anti-hepatitis B virus agents and their mechanism of action” (ESPS Manuscript NO: 20100). The manuscript has been carefully revised according to the reviewers’ comments. The responses to the comments were listed as follows.

For reviewer #1,

Minor Comment:

Major revision is required concerning language in term of grammar and structure.

Response: [The English language has been carefully revised.](#)

ABSTRACT

Fulfil the journal requirements. The aim of the work is clearly identified; however, a conclusion is better to be included

Response: [According to the above comments, this point has been revised.](#)

TEXT:

The section is almost well organized; an overall theoretical analysis concerning the provided data is well covered to great extent, however and in order to satisfy the reader, the authors are better to describe some important data regarding:

Citations regarding the problem of HBV worldwide (Ref.:1-5) are better to be updated. The provided data are not accurate for ref. No: 3.

Response: [According to the above comments, this point has been revised.](#)

Some important wards have to be fully written when mentioned for the first time ;e.g: DHBV, IC50, EMSA analysis

Response: [This point has been revised.](#)

The authors discussed the mode of action of several antiviral without identifying the level of studying the antiviral activity; *in vitro* or *in vivo* studies. This has to be maintained.

Response: [According to the above comments, this point has been revised.](#)

The antiviral activities of the illustrated agents are better to be mentioned in comparable to positive control.

Response: This point has been revised according to the statement of original references.

For reviewer #2,

The review manuscript written by Wu et al. describes various natural anti-HBV agents. Since HBV infection is still a serious problem around the world, the data are interesting and important. However there are some concerns that need to be addressed.

Major points 1. The most important problems of current treatment for HBV infection is that any anti-HBV agents has no effect on cccDNA, leading to relapse of HBV replication after discontinuation of the treatment. It is of note that the authors show some natural agents decreased cccDNA in the liver. If that is also true for human HBV infection, chronic HBV infection can be terminated in a drug-free manner. The authors should state more on the effect and mechanism of the action.

Response: Because it is very limited that the references regarding the natural products against HBV cccDNA. So I can not provide more information.

2. Emergence of treatment-resistant HBV mutants after long-term drug administration is another problem in HBV treatment. Long term antiviral effect of those natural agents should be shown.

Response: It is ture that the long-term use of the nucleoside analogues often result in drug resistance. Perhaps natural products is not easy to result in drug resistance. For example, Oxymatrine has been approved to treat the patients with hepatitis B in China for decades with a confirmed safety and often is used to replace the nucleoside analogues in order to decrease the emergence of treatment-resistant HBV mutants. Howerer, the natural products in this manuscript are almost in preclinical research stage. Therefore, we can not obtain more information on the long term antiviral effect of those natural agents.

3. Are there dose-dependent effects of each anti-viral agent?

Response: Yes, but the information of partial natural products come from their screening data.

4. Toxicities of each agent should be stated.

Response: These reported data were measured under nontoxic doses on cells or animals.

Minor point 1. HepG2.2.15 cells should have no cccDNA.

Response: It has been proved that there is HBV cccDNA in the HepG2.2.15 cells.

For reviewer #3,

Authors have prepared a good quality systematic review article. However, there are few modifications which needs to be done before commenting further.

- 1) Authors have not maintained the consistency in the formatting font while proof reading manuscript.

Response: According to the above comments, this point has been revised.

- 2) Spatial errors need to be corrected. For example, Line no. 17 of Introduction section etc.

Response: The English language has been revised.

- 3) Grammatical corrections need to be done by a native English Speaker. For example, Line 1 of Terpenes, Line 1 of Lignans etc.

Response: This point has been revised.

- 4) Botanical names of plants should be given in italics.

Response: This point has been revised.

- 5) In literature review, all IC50 values must be accompanied with their SEM value, if the same will be available in their parent manuscript which was cited by authors.

Response: Keep the same as the original reference.

- 6) If possible, authors must try to correlate structural features of these natural products with the activity defined, so that further optimization can be worked out.

Response: According to the above comments, this point has been revised.

- 7) In case of Magnolol mentioned in Lignans section, I have observed one article where magnolol and its virtual derivatives were docked with few target proteins of HBV. But such an information is missing in this article.

Response: [If possible, I hope you can provide this reference to me. Because I can not find it.](#)

- 8) Structure name

“4-Hydroxy-3-[2-(4-hydroxy-3-methoxy-phenyl)-1-hydroxymethyl-2-oxo-ethyl]-5-methoxy-benzald” given in figure 2 is incomplete.

Response: [This point has been revised.](#)

- 9) First time, authors have mentioned cccDNA in Terpene section. There only its full form should be mentioned, not in phenolic acids section. Authors are recommended to thoroughly check the manuscript for other discrepancy like this.

Response: [This point has been revised.](#)

- 10) Correction required in “ β -Thujaplicinol from the heartwood of Western Red Cedar trees inhibited RNaseHs from HBV genotype D and H in biochemical assays with IC_{50} values of 5.9 ± 0.7 and 2.3 ± 1.7 μ M, respectively” and “Six phenols m-hydroxybenzoic acid, p-hydroxybenzoic acid, m-hydroxy benzenmethanol, 3,4-dihydroxybenzoic acid, ethyl 3,4-dihydroxybenzoate” in Polyphenol section and “Therefore, it may be more feasible to obtain instrumental leads from naturally originated compounds with various skeletons and diverse biological activities than to directly screen drug candidates from natural products.” in conclusion section.

Response: [This point has been revised.](#)

- 11) Name of the assay protocol should be defined while illustrating any activity of natural products. So authors must cite the protocols with the help of which the activity of any molecule is reported.

Response: [This point has been revised.](#)

12) Reference list should be prepared according to journal's guidelines. Author can read some papers of journal for easy reference.

Response: [This point has been revised.](#)

Thank you for your checking and approving again. Please let us know if you have still any questions about our paper. We will answer your questions by all means.

Kindest regards,

Yi-Hang Wu