Peer Review File

Reviewer A

In this manuscript, the authors found that Paeonol induces anti-tumor effects in hepatocellular carcinoma cells through survivin via the cyclooxygenase-2/ prostaglandin E2 signaling pathway.

Major critiques:
Comment 1: The authors only used several HCC cell lines. More human HCC cell lines (Huh7 and Hep3B) were needed to verify the results.

Reply 1: This study is based on the Patient-Derived tumor Xenograft (PDX) model.

Comment 2: Control groups of none HCC specimens should be provided in figure 1a and b.

Reply 2: The control groups of the none HCC specimens have been provided in figure 2.

Comment 3: Control groups of 0 hour should be provided in figure 2a and b.

Reply 3: The control groups of 0 hour are shown as 0 in Figure 2e and 2f since there was no inhibition seen.

Comment 4: paeonol group should be provided in figure 6.

Reply 4: Paeonol group on survivin has been shown in other figures.

Comment 5: Annexin V binding are needed to support TUNEL assay data.

Reply 5: We agree with this observation, however, due to the COVID-19 pandemic, we have been unable to carry out the required experiments.

Comment 6: Additional pro-apoptotic factors (eg. FasL, TNFa, TRAIL) should be added to test the effect of Paeonol on death receptor mediated apoptosis.

Reply 6: We agree with this observation, however, due to the COVID-19 pandemic, we have been unable to carry out the required experiments.

Comment 7: Does Paeonol affect the expression of other mitochondrial proteins, for

example: Bcl-2, Bcl-XL, Bax, Bim, Bad, IAPs?

Reply 7: We agree with this observation, however, due to the COVID-19 pandemic, we have been unable to carry out the required experiments.

Comment 8: Expression of Survivin and COX-2 in cancer tissues of 10 patients and their corresponding noncancerous mucosal tissues should be analyzed by IHC or WB.

Reply 8: Thank you for the suggestion. We have included the IHC analysis of 10 patient tissues.

Comment 9: Tumorigenesis experiment should be made in nude mice.

Reply 9: Further study will be based on the Patient-Derived tumor Xenograft (PDX) model.

Comment 10: Poor writing and experiments design. The results of this experiment are not reliable. The author needs to provide all the original data.

Reply 10: The manuscript has been edited by AME editors. The certification be provided in an attachment.

The submitted manuscript described the paeonol anti-proliferation effect through surviving via COX2/PGE2 signaling pathway. However, there are so many issues indicating that this manuscript is not ready for publication.

Major concern:
Comment1: The language of the draft is really difficult to understand, so the author can take the help from a native English speaker.

Reply 1: The manuscript has been edited by AME editors. The certification be provided in an attachment.

Comment 2: The abstract should summarize the contents of the paper in a concise, and the author should pay attention and rephrase the abstract.

Reply 2: The abstract has been rephrased to ensure conciseness of information while ensuring that the word count restriction of the journal has been adhered to.

Comment 3: It is really confusing that why paeonol could be a marker in HCC cells described by the last sentence of abstract.

Reply 3: We have changed this sentence to as to avoid ambiguity.

Comment 4: Resolution of all figures is too low for people who need better details and results.

Reply 4: We have adjusted the resolution as per the journal guidelines.

Minor concern:
Comment 1: The abbreviation used in the draft should be defined upon first mention, such as COX2.

Reply 1: We have corrected this.

Comment 2: Please notice the latin abbreviations for plants.

Reply 2: We have corrected this.

Comment 3: The author should provide the structure of paeonol and other information, eg: the source, purity and NMR data.

Reply 3: We have now provided the source and purity of the paeonol used, and the molecule structure of paeonol has been provided as figure 1.

Comment 4: The description of paeonol concentration should in micromole (μM).

Reply 4: We have changed this as required.