Title: Chebulagic acid from Terminalia chebula causes G1 arrest, inhibits NFκB and induces apoptosis in retinoblastoma cells

Authors:

Naresh Kumar (naresh171@gmail.com)
Geetika Gupta (geetikagupta24@gmail.com)
Roy Karnati (roykarnati@gmail.com)

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Author's response to reviews: see over
Reviewer's report:

REVIEWER’S COMMENTS
This study by Kumar et al puts forward an alternative therapy for retinoblastoma. On the whole the results advocate the use of CA for the treatment of retinoblastoma and its cell sparing effect towards normal epithelial cells. Using the track change method I have highlighted the changes/comments in the manuscript.

Authors’ response: We appreciate the reviewer’s comments on the importance of our findings. The changes and comments highlighted in the manuscript were addressed and were incorporated in the revised manuscript.

1: There is no mention of the source of CA
Authors’ Response: CA was isolated from dried fruits of *Terminalia chebula* and the reference for the isolation procedure is included in the revised manuscript.

2: In the Western blot analysis, it appears there is complete absence of Bcl2 at 48 and 72 hrs, although the authors state it is a time dependent decrease.
Authors’ Response: The sentence was modified in the revised manuscript and it was stated to be completely absent.
Answers to comments : Referee 2

Major Compulsory Revisions

We appreciate the reviewer’s comments on our findings. These comments helped us to improve the manuscript.

1. The pathway Figure 8 is not reasonable.
   **Authors’ Response:** We appreciate the reviewer’s critical comments on the figure. We have modified the sequence of events and the interdependency between events in the pathway figure 8 (Figure 9 in revised manuscript) in the revised manuscript.

   - First, was decreased BAX/Bcl-2 in the downstream of increased cytochrome c release? Did author have any evidence?
     **Authors’ Response:** Upstream activation of BAX and inactivation of Bcl-2, BH3- only proteins oligomerize in the mitochondrial outer membrane and facilitate release of cytochrome C into the cytosol. We noticed the same; the sequence error in the figure is now corrected.

   - Second, no DNA fragmentation data (by gel analysis) in this manuscript. (only subG1 can’t summarize it).
     **Authors’ Response:** DNA fragmentation analysis with CA treatment by agarose gel electrophoresis is now included in the revised manuscript.

   - Third, how did increased p27 lead to DNA fragmentation? (p27 is a CDK inhibitor, not DNase).
     **Authors’ Response:** We rightly agree with the reviewer that increased p27 leads to cell cycle arrest but not DNA fragmentation. The figure 8 (Figure 9 in revised manuscript) is corrected in the revised manuscript.

   - The 4th, did decreased p65 cause cell cycle arrest? (Using p65 inhibitor showed the same effect?)
     **Authors’ Response:** p65 activates genes involved in proliferation of Y79 cells. Decrease in p65 did not cause cell cycle arrest. Decreased p65 causes decrease in proliferation of Y79 cells. The figure 8 (Figure 9 in revised manuscript) is changed accordingly in the revised manuscript.

2. It is necessary to analyze the effect of apoptotic inhibitor on CA-induced cell death. It can prove the importance of apoptosis in the death mechanism.

   **Authors’ Response:** In the view of your valuable comment, we checked for the role of Caspase 3 in CA induced cell death, Caspase 3 is activated in CA induced apoptosis. We have used Caspase 3 inhibitor, to show that CA induced cell death is mediated by Caspase 3 and is apoptosis. This data is incorporated in the revised manuscript.

3. In title it said, the apoptosis was induced via NF-kB. So, did apoptotic inhibitor also reverse p65 inhibitor-induced cell death?

   **Authors’ Response:** CA inhibited the translocation of p65. So this may be one additional property of CA in inhibiting the proliferation of Y79 cells. The title is the
manuscript and figure 8 (Figure 9 in revised manuscript) is changed more appropriately in the revised manuscript.

4. In “Methods - Cell culture” section, it should describe about normal human corneal epithelial cells. Where was it gotten from? How to culture?

Authors’ Response: It is human corneal epithelial cell line (HCE) available in ATCC. The culture conditions are described in Methods section of revised manuscript. The word ‘normal’ was deleted to avoid con