Reviewer's report

Title: Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential

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Reviewer: Kiran S Panickar

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Manuscript by Koppikar et al. entitled “Aqueous cinnamon extract (ACE-c) from the bark of Cinnamomum cassia causes apoptosis in human cervical cancer cell line (SiHa) through loss of Mitochondrial Membrane Potential”.

The manuscript by Koppikar et al., is interesting and an important contribution to increase the understanding by which cinnamon extract might induce apoptosis in a cancer line. These are carefully conducted studies and results reported might have a potential to be effective in vivo.

Several points need to be addressed to improve the manuscript.

Major compulsory revisions:

1. Mitochondrial calcium level was not measured in this study but the authors suggest that ACE-c increased calcium flux resulting into loss of mitochondrial membrane potential…. (page 13). While it is true that increased calcium is one of the inducers of the mitochondrial permeability transition (mPT), it is not the only one. Several of these polyphenol extracts also have pro-oxidant effects and it possible that the decline in the mitochondrial membrane potential was a result of the pro-oxidant effect of ACE-c. For instance by increasing nitric oxide (which also induces the mPT and thus a decline in mitochondrial membrane potential). The authors themselves have cited Ref #1 which shows a pro-oxidant effect of curcumin. Thus, there is no evidence that increased mitochondrial calcium was responsible for the decline in mitochondrial membrane potential although it is associated with it. This needs to be modified at several places (e.g. Abstract; Intro – page 4; Results page 13; etc). Alternatively, the authors could choose to measure mitochondrial calcium although this is not critical.

2. Cinnamon polyphenols were reported to attenuate the decline in mitochondrial membrane potential in C6 glioma induced by ischemic injury (Panickar et al., 2009; Experimental Neurology). In general, it seems many polyphenols either depolarize the mitochondrial membrane potential or prevents such depolarization. While not critical, it would be good if a small paragraph was added in the discussion that elucidates these contrasting effects seen with ACE-c or with polyphenols.
3. References 1-5 do not seem very appropriate for what the authors are trying to convey. Please cite appropriate references. As an example, the first sentence of the abstract of Ref #1 (Javvadi et al., 2008) is cited in this text but that is not the correct reference for what the authors want to convey in this manuscript.

Minor essential revisions:

4. There are several instances in the manuscript where the authors state “we have reported for the first time”. While these are interesting findings, such statements have no scientific merit and should be used minimally.

5. What is the concentration of JC-1 as well as Fluo3 used in this study?

6. Page 8 – In the immunoblotting procedure was the protease cocktail tablet used in addition to the aprotinin, leupeptin, pepstatin, etc??

7. In cell culture studies using the SiHa cell line, the authors should mention the passages used for the current studies. Several cell lines are known to behave differently when it exceeds certain passages. To compare results in the future, perhaps the authors can give some more details.

8. Repetition of “#” on page 10 (Statistical analysis).

9. This is just a suggestion but wouldn’t the flow of this manuscript be better if effects of cinnamon treatment on Her-2 expression in the Results section was moved immediately after the paragraph describing cinnamon’s effects on MMP-2 expression in the Results section?

10. Photomicrographs need scale bars.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I have published similar articles using cinnamon polyphenol extracts and its effects on neuroprotection but I do not have any conflicting competing interest.