Reviewer’s report

Title: Apigenin and hesperidin augment the toxic effect of doxorubicin against HepG2 cells

Version: 0 Date: 13 Jan 2019

Reviewer: Josip Madunić

Reviewer’s report:

The study by Korga et al. analyzed the effects of several flavonoids on the biology of hepatocellular carcinoma cell line HepG2, particularly in the context of cell viability and glycolytic gene expression. The presented data are interesting but this reviewer has some concerns that need better clarification by the authors.

Comments:

1. How do the authors explain that the combination of DOX with flavonoids other than apigenin or hesperidin, was not cytotoxic as DOX alone, as seen in Table 1? Could you please provide some theory behind this?

2. In the Results section on DNA oxidative damage determination, authors are claiming that they observed significant increase in AP sites in DOX-treated cells, and a drop in AP sites in DOX+A cells. These results (Figure 5) also show big SD deviation which brings in question the significance of authors' claims, especially in the case of A vs DOX+A which (when included the SD) could possibly be 4.28 vs 2.9. The authors should be more careful when assigning the significance.

3. Authors state that the MTT cytotoxicity assay was done three times. It should also be noted if the data in Figure 2. is mean value of these three independent experiments or their representative assay? Please correct this.

4. In the Results section, authors are over-emphasizing the effect of DOX and flavonoids on HepG2 morphology; other than the lower number of cells in the treated cell-panels, there are not apparent differences in cell morphology. This is not helped by the poor quality of images due to the excess of white spots (present in control and treated cells) which could be a consequence of over-exposure in imaging software and not due to the "presence of cytoplasmic vacuoles" as the authors are stating. Please elaborate on this. If the authors insist on these morphological changes, the use of arrows within the Figure 3 indicating them is then suggested.

Also, no magnification is mentioned and there are no scale-bars present in the Figure 3. Please provide. Figure 4 is also missing magnification and scale-bars.
5. In the Discussion section, authors state that the synergism of the cytotoxic effects of DOX and A is not related to the inhibition of glycolysis. Could you please elaborate on this in more detail?

6. Furthermore, authors suggest in the Discussion that combined administration of both agents - DOX and A leads to complete normalization of DNA oxidative damage and DSB. This is in contrast with the data presented in Figure 4. (panel DOX A), where one can observe plenty of CellROX signals indicating oxidative stress. The authors need to additionally clarify this before claiming that the oxidative damage by DOX is being abolished by apigenin treatment.

7. There are many misspelled words (i.e. Nicon/Nikon, StaftSoft/StatSoft, chemiotherapeutic/chemotherapeutic, etc), inconsistencies in verb tense usage, compound names (i.e. cosmosin/cosmosin), missing period and comma signs, as well as space between words, poor sentence constructs, etc.. Poor quality of writing does not seem to be a result of poor English, but more a consequence of negligence in manuscript checking. The authors need to check manuscript more carefully before submitting and the manuscript should be language-edited by a native speaker.

Minor revisions:

Title: „Apigenin and hesperidin augments the toxic effect" should be changed to „Apigenin and hesperidin augment the toxic effect"

Page 2, line 42: Please insert abbreviations for the hexokinase 2 and lactate dehydrogenase A

Page 3, line 4: Please change „of the couple of doxorubicin and apigenin" to more appropriate „of the combined (or synergistic) effect of doxorubicin and apigenin"

Page 4, line 35: Abbreviations for HK2 and LDHA should be given in full at the first mentioning in the text

Page 4, line 44: "higher than normal cells" should be changed to "higher than in normal cells"

The rest of the grammatical corrections is noted in the attached pdf of revised manuscript.

1. Last paragraph in the Introduction section is too long and not clear. I would suggest separating it in two sentences with clear emphasis on what was the aim of the research.

2. Authors are constantly using the term "inoculation" in the Methods section. I would like to point out that cells in cell culture are not "inoculated", they are "seeded". "Inoculation" in layman terms means introduction of something (i.e. tumor cells in xenografts experiments or pathogen/antigen in antibody production) into a living organism to stimulate something. Please correct this.
3. Many of the Figures are missing the description or just notion of the assay/method used for obtaining presented data. Please correct this where applicable.

4. Proper format of cited reference in the Discussion on Page 13, line 42 is "Vrhovac Madunić et al" (two last names). Same in the list of references.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

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I am able to assess the statistics

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