Reviewer's report

Title: Citrus auraptene suppresses cyclin D1 and significantly delays N-methyl nitrosourea (MNU) induced mammary carcinogenesis in female Sprague-Dawley rats

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Reviewer: Gallo Daniela

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The study from Krishnan and colleagues was aimed at evaluating the chemopreventive activity of citrus auraptene in mammary cancer. To this end, both in vitro and in vivo experiments were performed. In vitro results showed inhibition of MDA-MB-231, and to a lesser extent, MCF-7 cell proliferation. In MCF-7 cell line there was also a decrease in IGF-1-induced cyclin D1 expression. In vivo results showed that citrus auraptene (200 and 500 ppm in the diet) did not reduce the incidence and the mean number of MNU-induced mammary tumors/rat, while it delayed the development of tumors in the high-dose group (i.e. 500 ppm), possibly by decreasing the expression of cyclin D1.

The rationale of the study was reasonable, since in the last years a chemopreventive effect has often emerged for citrus auraptene, although in different cancer models; also, the design and the conduction of experiments appear to be of a good quality. This notwithstanding, the manuscript presents some important drawbacks that, in my opinion, make it unsuitable for publication. Here follows a list of the problems that should be addressed.

Major compulsory revisions

1. General comments. The manuscript is not carefully written, with data not properly presented and discussed, a condition that makes it difficult to determine the overall quality of the paper. The Discussion is not focused on the actual findings, and results should be analytically discussed in the light of other literature data on the compound. For example, other studies showed that the chemopreventive activity of citrus auraptene might be associated, at least in part, with increased activity of Phase II drug-metabolizing enzymes (Tanaka et al., Cancer Res, 1998; Tanaka et al., Carcinogenesis, 1998). Thus, besides cell proliferation, other mechanisms are likely to play a role in the observed effects, and this issue should be adequately addressed in the Discussion. Conversely, other parts of the Discussion should be removed, due to the lack of relevance (e.g. the 5th paragraph “Breast cancer like most other cancers….”).

Furthermore, some sentences should be rewrite: a) to improve clarity [e.g., page 9: "The rat weight data was compared between groups after calculating the area under the weight versus time curves for each rat by One-Way ANOVA followed by Fisher’s LSD test" (do the Authors refer to body weight or tumor weight data?)]
"Among" instead of "between" should be used for more than two groups. Does the calculation refer to each rat or to each group of rats?), or b) to improve the writing style [e.g. Page 14: "Based on the results of auraptene on the expression of cyclin D1 expression in MCF-7 cells, the tumors from the rats were analyzed for cyclin D1 expression". In addition, there are colloquialism (pages 8 and 28, “@” for “at”), and typos (e.g. page 9, first line, “expect” instead of “except”; line 5, “cylcin” instead of “cyclin”, etc.).

2. Materials and Methods/Results. It would have been reasonable to analyze citrus auraptene concentration in tumors in Auraptene 500 ppm group, to possibly correlate findings observed with tissue concentrations. Why did the Authors choose not analyze tumors in the high-dose group? In addition, Authors should explain the reason for choosing different sample sizes for HPLC analysis of citrus auraptene concentration in rat tissues (i.e., 5, 8, 2, 7, see Figure 9).

3. Materials and Methods/Results. Auraptene liver concentration was determined in only two samples: mean ± SE cannot be used for such a low sample size. In addition, the mean value reported in the Results (i.e. 0.87 ± 0.37 uM) does not appear to reflect the mean of the two individual values reported in Figure 9. This value should also be eliminated from the “Abstract”.

4. Results. It is not clear why the Authors decided to analyze tumor data at both week 16 and 18.

5. Results. The Authors claimed that “…..the total numbers of tumors were found to be significantly reduced in MNU/Auraptene 500 ppm group animals when compared to MNU only group (p< 0.05).”. Please report the relevant numbers.

Minor, essential revisions


3. Material and Methods. Page 6. “Cell Proliferation Assay” paragraph: only the MDA-MB-231 cell line is mentioned, while no indication is reported in relation to MCF-7. Please add.

4. Materials and Methods. Page 7. “Treatment of rats” paragraph: Figures should be in the order in which they are described in the text: i.e., the text discusses Figure 4 before Figures 1 -3.

5. Material and Methods. Page 9. Please clarify if the membrane used was nylon or PVDF.

6. Materials and Methods. Page 9. Please give an explanation for the choice of the post-hoc multiple comparison test. The Least Significance Difference (LSD) Test is, in fact, generally not recommended, since this procedure emphasizes sensitivity (minimizing type II errors) but at the possible cost of increasing type I errors.
7. Results. Data in Figures 9 (Concentration of Auraptene in rat tissue) and 10 (Effects of dietary Auraptene on cyclin D1 expression in rat mammary tumors) do not match comments in the Results section. Please check. Also the relevant figure legends need to be rectified.

8. Figure 9 – Values on the X axis should be properly reported.

Discretionary revision

1. Material and Methods. Page 7. Figure 4 can be eliminated since the relevant information is already reported in the text.

2. Material and Methods. Page 9. The reason for using a range of 4 to 20% polyacrylamide gel for western blot analysis of two proteins with a comparable molecular weight (i.e. actin 45 KDa and cyclin D1, 36 KDa) should be explained.

3. Material and Methods. Page 9. It is not clear why the Authors refer to the Yin et al. paper (#26) to describe methods for western blot: it would be better to add details of procedures used for protein extraction, and eliminate reference #26.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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

Declaration of competing interests:

I declare that I have no competing interests