Re: BMC Complementary and Alternative Medicine submission
Oral Squamous Cell Carcinoma Proliferative Phenotype is Modulated by Proanthocyanidins: A Potential Prevention and Treatment Alternative for Oral Cancer

Enclosed please find the revised manuscript *Oral Squamous Cell Carcinoma Proliferative Phenotype is Modulated by Proanthocyanidins: A Potential Prevention and Treatment Alternative for Oral Cancer*. This manuscript presents new data exploring the relationship between proanthocyanidin, an over-the-counter plant-based extract, and reduced proliferation of oral cancers with the first evidence of PAC-induced proliferation inhibition among oral cancers infected with the human papillomavirus.

We have addressed all of the major compulsory revisions, minor essential revisions, and the discretionary revisions, which were suggested by the reviewers. Separate, individual rebuttals for each critique are detailed on the following pages, which are summarized by the following revisions:

- Inclusion of numeric (quantitative) evidence to support conclusions
- Revision of proliferation graphs and figure
- Inclusion of source and composition of proanthocyanidins used in study
- Incorporation of virtually all ‘wordsmith’ recommendations and suggestions

We greatly appreciate the suggestions and input from the reviewers and strongly believe that this manuscript addresses new and important results regarding the potential use of proanthocyanidins in the treatment of oral cancers of interest to physicians, cancer researchers and other readers of BMC Complementary and Alternative Medicine. We would like to thank you in advance for your consideration of this manuscript and look forward to your response. Please feel free to contact us at your convenience with any comments or questions.

Sincerely,

Karl Kingsley, PhD
Assistant Professor of Biomedical Sciences
Critique 1:

Major Compulsory Revisions:

- **No further experimentation was required; however, the reviewer suggested that the inclusion of numeric evidence would help to support our conclusions.** We concur with these comments and to have amended this manuscript with quantitative, numeric observations of cell viability, cell spreading and confluence of each cell line, in the presence and absence of PAC (Table 1). These data suggest that PAC induces a pronounced reduction in viability and spreading of cancerous cell lines, but not the normal cell line.

- **Although the reviewer did not believe that further experimentation for this manuscript was warranted, it was suggested that assays for apoptosis markers could be run.** We believe that our results from this manuscript suggest the possibility of apoptosis and advocate for the elucidation of this mechanism in future studies.

Minor Essential Revisions:

- **Suggested revisions:**
  - Page 3: PAC-induced proliferation, inhibition, and apoptosis…
  - Page 8: seeded in 25 cm2 BD Falcon…
  - Page 12: cell lines (Fig. 1C, 1H). The….

- **We concur with these suggestions and have modified the text to include these changes.**

- **Suggested revisions:**
  - ….non-transfected CAL 27 (Fig. 1D, II). Some….
  - We have modified the text to include these changes as follows: “CAL 27-TF16 cells (Fig. 1D) demonstrated…” and “….in the presence of PAC (Fig. 1I)…”

  - ….and blebbing at day 4 (I), while….  
  - We have modified the text to include this change as follows: “CAL 27-TF16 (Fig. 4I), were more resistant…”

  - …the majority of GH354 cells (G) and….  
  - We have modified the text to include this change as follows: “…the majority of GH354 cells (Fig. 4G)…”

  - ….non-transfected CAL 27 cells (H) had…
  - We have modified the text to include this change as follows: “….non-transfected CAL 27 cells (Fig. 4H) had….”
Discretionary Revisions:
- Suggested revisions:
  - Page 11: An overall reduction...number of cells observed – rewrite as proportionality. We concur with this suggestion and have modified the text as follows: “...was a proportional reduction....”
  - Figure 2 A,C, E, G and I are confusion – suggestion to distinguish the curves. We concur with this suggestion and have modified these sections of Figure 2 to include only the most relevant curves (0, 10, 20, 50 and 100 µg/mL PAC), as well as a key which enables the reader to more easily identify and distinguish these trends. All of the data (concentrations including 0, 10-100 µg/mL PAC) are given in Fig. 2B, D, F, H and J for further clarification, if necessary. In addition, we have labeled GI\text{MAX} for each cell line in each of the appropriate sections.

Critique 2:

Major Compulsory Revision:
- The authors do not cite the produce number or type of PAC used...it is important that they do so. We concur with these comments and to have amended this manuscript in the Methods section with “Materials”, which specifically indicates the source (GNC Preventive Nutrition) and lot number (3717HF7361) of PAC used. Furthermore, we have clarified the plant source of this PAC (\textit{Vitus vinifera}) and included information regarding PAC composition, with quantitative, numeric observations of cell viability, cell spreading and