Reviewer’s report

Title: Oral administration of PPC enhances antigen-specific CD8+ T cell responses while reducing IgE levels in sensitized mice.

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Reviewer: Tong-Rong Jan

Reviewer’s report:

This article demonstrates that an extract of pine cone suppresses IgE production and modulates T cell-mediated immune responses in vivo. The work seems to be adequately performed and the results appear convincing because the authors use several parameters to study the immunomodulatory effect of the extract. Although the main idea of the paper is interesting, there are some points to consider:

Major points:

1. The effective doses of PPC shown in Figures 1-5 are 200 and 2000 microgram/mL, whereas lower doses were used in experiments conducted in aged mice. What is the rationale to compare the effects between different doses under different experimental conditions?

2. Although the authors describe in the Introduction section that systemic absorption may occur after oral administration of PPC. It is unclear if the plasma concentrations reach 200 microgram/mL after taking PPC in humans. The concentrations used in the present studies will have to be justified.

3. The presented results appear quite diverse, including humoral responses (Figures 1-3), cytokine production (Figures 4-6), and CD8+ T-cell responses (Figures 7 and 8). Although the results of Figures 7 and 8 confirm the enhancing effect of PPC on CD8+ T-cell responses presented in Figure 4, these data are not directly related to allergy, and therefore not to fit with the main idea of conducting the research based on the improvement of allergic symptoms in consumers taking the pine cone extract. The paper would be strengthened if the effect of PPC on allergic inflammatory reactions (i.e. airway hypersensitivity reactions) other than IgE production was studied.

4. For studying the effect of PPC on the Th1/Th2 immunobalance, an investigation on the production of IgG subtypes (i.e. IgG1 and IgG2a) induced by an allergen (i.e. ovalbumin) would provide a direct evidence to complement the cytokine results. However, Figure 3 shows only IgG2a. It will be more convincing if IgG1 is included in the study.

5. A quite extensive discussion on the development and differentiation of Th1 and Th2 cells was included in the Discussion section (page 19). However, the authors did not present data on the influence of PPC on Th cell differentiation, such as the signaling transcription factors T-bet and GATA-3 for Th1 and Th2 cells, respectively.
6. Are the data presented in each figure derived from one single experiment? How many times of each experiment are repeated? The authors should clarify this issue.

Minor points:
1. For all IgG2a, 2a should change to subscript word.
2. For all CD8+, + should change to superscript word.
3. For the word “ug”, the u should change to the abbreviation of "micro".
4. For hr, hours and hrs, ml and mL, and ul and uL, standard abbreviations should be followed.
5. For the description of cell concentration (i.e. 3-5x106/mL on page 9), it should change to 3-5 x 106 cells/mL. The entire manuscript has to be checked for consistency.
6. On page 5: 0.2um should change to 0.2 um.
7. On page 5: 11.3k should change to 11,300.
8. On page 6: “+- either” should be rewritten.
9. On pages 6, 7 and 9: Day should change to day.
10. On page 10: Avidin-HRP should change to avidin-HRP.
11. On page 10: PBST and AEC substrate should be specified.
12. On page 12, line 3 from the bottom: were should change to was.
13. For all figures: do error bars represent standard deviation?
14. Statistic significance should be indicated in all figures.
15. Figure 2 legend: Day should change to day.
16. Figure 2 legend: 100ug should change to 100 ug.
17. Figure 2 legend: CPG should change to CpG.
18. Figure 5: the IL-4 level in the OVA+PPC group is less than that in the non-sensitized Naive group. This will have to be explained.
19. Figure 7 legend: ovalabumin should change to ovalbumin or OVA.

**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:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.