Author's response to reviews

Title: A water extract of Brazilian green propolis has a laxative effect via activation of acetylcholine receptor

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Author's response to reviews: see over
Responses to reviewer’s comments

Reviewer #1

Major revision:
I have pointed out the problem about the manuscript and it still remains in the revised manuscript. The authors should identify the bioactive compounds included in the WEP and clarify the molecular mechanism. These are the most important purpose. I recommend the authors demonstrate these issue and resubmit your manuscript as a new version to our journal.

We think molecular mechanism is very important, but we are going to publish our studies one by one and step by step. As Professor Tsuda mentioned, our paper failed to clarify molecular mechanism, but we have hardly seen such herbal medicine that induces clear increment of isolated ileum tensions in magnus method like WEP. We think this study is worth enough to be published from this viewpoint.
Reviewer #3

1. The work have been done with two kinds of extracts but the title mentions only. In the other I am not agree with the formulation of the title. My proposal is: “Laxative effects and mechanism of action of Brazilian green propolis”. We changed the title of our title according to the reviewer’s proposal (p.1-L.2).

2. The authors should give the range of the tested doses of extracts in the abstract. We added the concentrations of the administrated propolis (p.2-L.10).

3. In the results section, line 2: authors should indicate the p value for the significance of results. The statistical program J-STAT does not indicate individual p-values in multiple comparison test; it indicates * as p<0.05 and ** as p<0.01. We added “p<0.01” in the results section (p.2-L.16).

4. In many reports, caffeoylquinic acids are recognized as the major active constituents. The authors should add some references. We added references (p.4-L.19).

5. The EEP and WEP had much stronger antioxidant activities against all types of reactive oxygen species (ROS) when compared to the activities other bee products, such as royal jelly and bee pollen [25]. The authors should insert “of between activities. We inserted “of” at the indicated position (p.4-L.21).

6. Constipation is a symptom rather than a specific disease. It has many causes, including chemical compounds (e.g., morphine, clonidine, etc.), dietary habits (e.g., low-fiber diet, low-vitamin diet, high-fat diet, high-protein diet, etc.), composition of intestinal flora, pregnancy, and psychological stress. The authors should add references here. We added references (p.5-L.13).

7. The effect of propolis on constipation has not been previously reported, but its use in traditional medicine indicates that it may have a laxative effect. The
authors should indicate the traditional uses in relation with laxative effects.

A use of propolis as laxative is only traditional, folk, oral, and not reported. We cannot show academic references, because we are the first one that shows scientifically reported laxative effect of propolis.

We can show public advertisement (http://hedera.hr/index.php/propolis-effects/digestive-system/), but it has been an only scientifically groundless information until this paper is published.

8. In the extraction procedures of EEP and WEP, authors should say how they obtained the 12g of EEP and the 10g of WEP (evaporation lyophilisation…?)

We added the method we used to gain each extracts (p.6-L.15 and L.20).

9. In the Animals and ethical approval section, authors should tell the weight of mice and guinea-pig.

We added the approximate body weights of mice and quinea-pigs (p.7-L.4 and L.5).

10. In the gastro-intestinal (GI) transit section, authors should replace WEA by WEP.

We replaced the “WEA” with “WEP” (p.8-L.16).

11. In the statistical analyses section, the authors should indicate the p value for what the compared data are significantly different.

The statistical program J-STAT does not indicate individual p-values in multiple comparison test; it only indicates * for p<0.05 and ** for p<0.01 as a result of Dunnett’s multiple comparison tests. We added “*p<0.05, **p<0.01” in the statistical analyses section (p.10-L.10 and L.11).

12. The Fig 2 (page 20) showing the experimental protocol for the constipation models should be deleted because they are not necessary.

We deleted figure 2 as reviewer suggested.

13. The authors should indicate the number of animals and groups used for all experiments.

We indicated the number of animals (isolated ileum) and groups in each paragraphs (p.7-L.15, p.8-L.4, p.8-L.15, p.9-L.6, p.9-L.19, p.10-L.1, and p.10-L.3).

14. The authors should delete “We first investigated the effects of WEP and EEP
on stool weight in normal mice”, “We induced constipation by two different mechanisms to investigate the biochemical mechanism of the laxative effect of WEP: via the \( \mu \)-opioid receptor and via the \( \alpha-2 \) adrenergic receptor”, “We investigated the effect of WEP on stomach and jejunum by measuring GI transit”, and “We investigated the effect of WEP on ileum and intestinal tension by measuring the tension of the isolated ileum in guinea pigs (Fig. 4B, C, and D). WEP was cumulatively added to the ileum suspended in an organ bath of the Magnus system and tension was monitored every 0.5 second).”

We deleted all the sentences according to the reviewer’s suggestion.

15. The author has to state clearly on the pharmacological effect of the major compounds to know caffeoylquinic acids. Why this acid is the major compound in the two extracts and they have not the same laxative effect? He can search bibliographic informations concerning the other metabolites presents in the two extract and find the one responsible of the laxative effect of the water extract as an hypothesis to verify with other appropriate tests.

We already mentioned di-CQAs (caffeoylquinic acids) in the result, but our discussion is not sufficient. We think that di-caffeoylquinic acids and there metabolites cannot be active compound because they contain in the two extracts.