Reviewer's report

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

Version: 3 Date: 3 August 2012

Reviewer: J. David N'guessan

Reviewer's report:

1) Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

* Title:
-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”.

2) Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

* In the abstract:
-The authors should give the range of the tested doses of extracts in the abstract.
-In the results section, line 2: authors should indicate the p value for the significance of results.

* In the background line 33:
-In many reports, caffeoylquinic acids are recognized as the major active constituents.
The authors should add some references.
-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 and other so that the sentences will be:
“The EEP and WEP had much stronger antioxidant activities against all types of reactive oxygen species (ROS) when compared to the activities of other bee products, such as royal jelly and bee pollen [25]”

- 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.

- 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.

* In the Methods
- 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…?)
- In the Animals and ethical approval section, authors should tell the weight of mice and guinea-pig.
- In the gastro-intestinal (GI) transit section, authors should replace WEA by WEP.
- In the statistical analyses section, the authors should indicate the p value for what the compared data are significantly different.
- The Fig 2 (page 20) showing the experimental protocol for the constipation models should be deleted because they are not necessary.
- The authors should indicate the number of animals and groups used for all experiments.

* In the results
General remark: The writing of this section must be improved.
- Effects of WEP and EEP in normal mice
The authors should delete “We first investigated the effects of WEP and EEP on stool weight in normal mice” and go straight to the results.
- The effect of WEP on stool parameters in two types of constipation model mice
The authors should delete “We induced constipation by two different mechanisms to investigate the biochemical mechanism of the laxative effect of WEP: via the µ-opioid receptor and via the #2 adrenergic receptor.
Effect of WEP on GI transit
The authors should delete “We investigated the effect of WEP on stomach and jejunum by measuring GI transit”.

Effect of WEP on the tension of isolated ileum of guinea pigs
The authors must delete “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). This details should be given in the method section.
3) Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

- In the discussion

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.