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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Version: 3 Date: 31 May 2012

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

Reviewer #1

1. The data is lacking molecular mechanism of the effect. Crude extract should not be used to demonstrate these effect in vivo and vitro system. What are the most bioactive compounds in the WEP? There is no analytical data of components in the WEP.

   I agree that we need viewpoint from molecular mechanism in the effect of WEP. According to reviewer’s advice, we added some sentences in discussion (p12-L17 to p13-L11).

   The main purpose of the present study was to investigate potential laxative effects of WEP scientifically. We added a sentence in discussion (p12-L9 to L10). As the reviewer suggested, decision of active chemical constituents and molecular mechanism are very important, but we would like to do it as the next theme.

2. WEP concentration is too high. Does the authors think the high dose of WEP is significant important? Will unknown constituents distribute to the target tissues and give significant impact to cells? Overall, the experimental data using high-dose of crude extract with unknown constituents is not worthwhile for readers.

   The reviewer indicated that crude extract is unsuitable to our study in vivo and in vitro (ex vivo), and our dose (10, 50, 100, and 500 mg/kg) is too high.

   We used crude propolis extract in the present study because human consume propolis as extract of ethanol or water. Dai-Kenchu-to, one of the most typical herbal medicines, presented laxative effect at 1000 mg/kg in an animal experiment and at 1000 µg/ml (we examined less than 300 µg/ml) in magnus method [Jpn J Pharmacol., Abatement of Morphine-Induced Slowing in Gastrointestinal Transit by Dai-kenchu-to, a Traditional Japanese Herbal Medicine, 2002 Feb;88(2):217-21.].

   In the journal BMC CAM, Mareya micrantha aqueous extract showed laxative effects at 400 mg/kg [BMC Complement Altern Med., Laxative activities of Mareya micrantha (Benth.) Müll. Arg. (Euphorbiaceae) leaf aqueous extract in rats., 2010 Feb 16;10:7.].

   We recognize that propolis shows some effects at 50 mg/kg (high-fat induced model, Koya-Miyata et al.), 200 mg/kg (streptozotocin model, El-Sayed et al.), 10 mg/kg (antihypertensive effect, Maruyama et al), and so on, meaning lower doses than we examined in the present study. But as to laxative effect, propolis needed a higher dose (500 mg/kg) to show significant effects, and we think this dose is not too high among other herbal medicines (Dai-Kenchu-to, at 1000 mg/kg; Mareya micrantha, at 400 mg/kg; Aloe ferox, at 200 mg/kg etc.).
Reviewer #2

Major revision:
1. The authors show that the water extract shows laxative potential and not the ethanol extract. Please discuss in a little detail, in the discussion section, as to which of the known chemicals (in the water extract) might be responsible for the observed effects (support your argument by using available references in the literature)

   We added the sentences of explanation in discussion as the reviewer’s suggestion (p12-L17 to p13-L11).

2. The water extract reverses clonidine induced constipation but not loperamide induced constipation. The authors have given a bit of argument in this regard in the discussion but this is not enough, please elaborate!

   We added the discussion on several autacoids which have influence on contraction of intestinal smooth muscle (p13-L13 to p14-L9; p14-L21 to p15-L13).

3. It is very unusual for a substance that is showing increased stool formation, reversing clonidine induced constipation, and has direct stimulant effect on isolated intestinal jejunal smooth muscle preparation to not have any significant stimulant effect on intestinal transit? Please explain!

   We partly confused “jejunum” and “ileum” in our paper. We used “ileum”, meaning that latter part of small intestine, in magnus method. We added the sentences of explanation (p16-L3 to p16-L11).

Minor revision:
Background, last paragraph: the authors say that propolis has a traditional use as a 'laxative' agent; please give a reference for this claim!

   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 only scientifically groundless information until this paper is published.