Author's response to reviews

Title: Effect of Tualang honey on the healing of anastomotic wound in large bowel anastomosis in rats - A randomized controlled trial

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Author's response to reviews: see over
COVER LETTER

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Dated: 29/10/15

Dear Editor BM C (C & AM ),

I wish to submit a revised manuscript entitled "Effect of Tualang honey on the healing of anastomotic wound in large bowel anastomosis in rats - A randomized controlled trial" for consideration by BM C Complementary & Alternative Medicine journal.

With the submission of this revised manuscript, I would like to address the questions from the reviewers point-by-point as follows:

Reviewer: Scott Sell

Major revisions:

1. Manuscript has been revised carefully for all the glaring grammatical errors, hope it is now in its best shape for publication requirements.

2. Requested references in the background section regarding use of various healing agents (i.e. honey, aloe vera and snakhead fish (haruan)) have been provided. (Shan et al. for Aloe vera and Jaiz et al. and Mustafa A et al. for Haruan. See references.)

3. Regarding the duration of the study, it was conducted for seven days only because it is based on physiological phase of wound healing during which the cell proliferation and matrix deposition phase starts, including fibroplasia, angiogenesis and re-epithelization. This type of study has been substantiated by previous literature by Aşlan et al. (Aşlan A, Temiz M, Hakverdi S, Polat G, Tumer C, Temiz A, et al. Effect of mesalamine on healing in experimental colonic anastomosis: A randomized experimental study. International Journal of Surgery. 2008;6(1):40-4.) and Yole et al. (Yole S, Tekin A, Yılmaz H, Küçükkartalar T, Esen H, Çağlayan O, et al. Effects of platelet rich plasma on colonic anastomosis. Journal of Surgical Research. 2008;146(2):190-4.). Furthermore, this is an initial experimental stage study and the idea of looking at the healing process at multiple time points has been noted and may be taken into account for future studies.

4. Regarding the dose of Tualang Honey (TH) used, it was based on the suggestion by Aljadhy et al. (2000) which concluded that the best results on wound healing could be obtained by giving 1.5-2.0 ml orally plus 0.1-0.15 ml/cm² topically or by topical application of 0.2-0.25 m/l/cm². Due to an
experimental nature of our study, we used lesser dose of TH as compared to another local Malaysian honey (Apis mellifera honey) used in the study by Al-jady et al., which was hypothesized to be less potent than TH and so, optimal dosage evaluation for TH is a research area for future studies.

5. Collagen matrix production was not assessed in histopathological evaluation due to our cost limitation, only cell proliferation phase was examined at the day seven.

6. We didn’t use an intact, non-operated control because the comparison was being done in subjects which underwent anastomosis and the tensile strength was assessed after the procedure. So, we used operated animals without honey administration as control.

7. Hematoxylin and Eosin stains were used and we did assessed the Fibroblast count, inflammatory cells and blood vessels collectively and were counted semiquantitatively and graded histologically in a blind fashion, using the 0–4 Ehrlich and Hunt numerical scale (Ehrlich et al. in references)

Minor Essential Revisions:

1. About the surgical protocol animal model, two studies (Aslan et al. & Yole et al.) were used to develop our surgical protocol and animal model. (see references)

2. A study from Aslan et al. (2008) was our reference for the bowel burst strength testing technique.

3. The pressure was applied by injecting room air in a container filled with saline and colon segment inside it and increased constantly at a rate of 5m l/minute through an infusion pump. Endpoint was a sudden decrease in the pressure or when bubbles were seen.

4. All the results were presented as mean with standard deviation (SD).

Reviewer: Jaroslava Halper

1. How did they decide to euthanize all rats on Day 7, and why only one data point was used?

   Answer same as major revision’s bullet point question number 3.

2. Did they attempt to do other types of biomechanical testing (e.g., for elastic modulus)?

   No, we did not attempt for elastic modulus (limitation of study).

3. I assume that histology sections were cut from tested tissues?

   Yes.

4. How many samples were tested for biomechanics and how many for histology and cell counts?

   All animals in the study were tested for biomechanics and histology.

5. The main problems are in the histology and its evaluation. It is not clear what these cells counts of fibroblasts and inflammatory represent. In other words, did they count cell per field (at what magnification), and if yes,
how many fields, or several m², or a portion of the slide (this should be in Methods)? In all layers of the colon? What inflammatory cells they saw (hard to tell from the provided photo)?

The anastomoses were graded histologically in a blinded fashion, using a modified numerical scale according to Ehrlich and Hunt (E&H) (reference added). Inflammatory cell infiltration, vascular ingrowth, and fibroblast proliferation were graded, that is, 0 (absence), 1 (occasional presence), 2 (slightly distributed), 3 (abundance) and 4 (confluence of cells and fibers). Cells were counted in 10 fields in the random fashion and the average was taken. Magnification of 40x was used. Yes, cells were counted in all layers of colon. Inflammatory cells such as lymphoplasmacytoma cells, macrophages and polymorphonuclear leucocytes were seen. They were counted semi-quantitatively and graded as said before using 0-4 E&H scale.

6. And if more than one, what types and they should count how many of each type they found. They should also evaluate the presence of newly led collagen or at least of connective tissues, esp. if they mention this neoformation on the bottom of p. 9 (Discussion). Immunohistochemistry for type I and III collagen, or at the very least trichrome and reticulin stains should be provided.

We assessed neoformation of vascular tissue only and not the newly led collagen. It is the limitation of the study due to cost.

Please address all correspondence concerning this manuscript to me at andee778@gmail.com

Thank you for your consideration of this manuscript.

Sincerely,

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