Reviewer's report

Title: Topical Green Propolis Improves Corneal Wound Healing and Inflammation in Rats following Alkaline Burns

Version: 1 Date: 22 May 2013

Reviewer: Luciola Barcelos

Reviewer's report:

Martin et al. proposed to evaluate the effects of topical Brazilian green propolis in alkali-burned cornea wound healing and inflammation. They observed propolis reduces the area of corneal epithelial defect and the inflammatory cells count in the injured area, although does not alter the pattern of leukocytes migration to the center of injury. In addition, it was observed propolis increases the proliferation of epithelial cells. Authors conclude that topical Brazilian green propolis accelerated wound healing and reduced inflammatory response after silver nitrate-induced alkali burns in rats.

Major Compulsory Revisions:

1. It was already known that topical propolis has an anti-inflammatory effect on alkali-injured eyes (The Effect of Propolis Extract in Experimental Chemical Corneal Injury. Öztürk F et al. Ophthalmic Res 2000;32:13–18 (DOI: 10.1159/000055581)) and authors must not only reference this work but also discuss the differences (if any) between their finds and/or model used and that one that could characterize novelty in this aspect of their work;

2. Regarding the inflammation analysis, authors should give detailed information about the parameters that were used to identify the leukocyte infiltrate as well as a more accurate explanation if they performed total and/or differential counts of leukocytes. None of those information is stated anywhere. In fact, in the Material and Methods section they claim they count “leukocytes” (it’s not clear if total or not) and, in the Table 2, the data is presented as “neutrophil” count;

3. Regarding leukocyte migration analysis in situ, I’m not convinced about the accuracy of the methodology the authors have used. If this is a standard methodology to access this parameter on corneal injuries, authors should give a reference for this method. Considering that there is no newly formed vessels at the time points evaluated and that the cornea is an avascular structure and so that leukocytes should come from vessels from other regions than cornea at those time points, the leukocytes present in the borders, but not necessarily in the center of the wound, would already represent cells that have been migrating. In addition, it’s not clear why authors count leukocyte migration (that I’m considering as total leukocyte count) as “center/total ratio” and subsequently they consider count neutrophils “center/border ratio” as a second parameter of leukocyte migration. Which is the difference between those “parameters”? Also the description of this analysis is very confuse and should be revised;
4. As propolis chemical composition, that influences its activity, depends not only on the phytogeographic characteristics of the place of collection, but also on the solvent used during the extraction process and the information about which solvent was used during the extraction is not stated anywhere in their work, authors should describe it on Material and Methods section;

5. Still regarding propolis chemical composition, what are the most bioactive compounds present in the extract used in the present work? There is no analytical data of the components of the extract used in the present work or cited reference for that. This information should be given.

Minor Essential Revisions:

1. Authors should consider display representative images of fluorescein-stained eyes and representative H&E images of histological sections of corneas after injury for illustrating their finds on differences between green propolis and vehicle regarding wound closure and inflammation, respectively;

2. If authors choose for showing differential leukocyte count (instead of total leukocyte counts) at wound sites, they should be more accurate in the naming of cells as polymorphonuclear and mononuclear leukocytes as any specific analysis (e.g. immunohistochemistry using specific antibodies) was done to state which cell type was exactly present at wound sites;

3. Authors should state in the Material and Methods section how many drops per eye per application and what was the volume of each drop used in their experiments;

4. Considering experimental animal ethics, authors should change the word “sacrificed” for “euthanized” everywhere;

5. The description of “wounded area” result should be better elaborated as that reader could have a clear notion that propolis reduces the area of wounds.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests