Author's response to reviews

Title: Morus alba L. suppresses the development of atopic dermatitis induced by the house dust mite in NC/Nga mice

Authors:

Hye-Sun Lim (qp1015@kiom.re.kr)
Hyekyung Ha (hkha@kiom.re.kr)
Hoyoung Lee (lhoyoung@kiom.re.kr)
Jun Kyung Lee (bi235@hanmail.net)
Mee-Young Lee (cozy11@kiom.re.kr)
Hyeun-Kyoo Shin (hkshin@kiom.re.kr)

Version: 3 Date: 11 March 2014

Author's response to reviews: see over
Manuscript number: 9307176671056554

Title: *Morus alba* L. suppresses the development of atopic dermatitis induced by the house dust mite in NC/Nga mice

BMC Complementary & Alternative Medicine

Dear Editors

Thank you very much for your editorial decision letter, which also included the reviews of our manuscript by referees. We have made the changes as suggested by the reviewers. The changes are marked in blue in the revised text. We have made during the revision in a point-by-point response to each of the comments.

We hope the revisions made the responses provided are satisfactory, and our manuscript is now acceptable for publication in the BMC Complementary & Alternative Medicine.

Please, let us know if further revisions are needed.

Once again, thank you for all your help. We look forward to hearing from you.

Sincerely yours,

Hye-Sun Lim, Ph.D.
qplhs1015@gmail.com
Reviewer #1:

1) The abbreviations must be mentioned when they first appear in the text, many times the abbreviations are repeating, but these might not be a significant error for the work contribution to upgrade the knowledge. I think the manuscript has to be rechecked for this type of smaller errors.

   We appreciate your comment. We thoroughly checked manuscript as commented by reviewer.

2) I was wondering what is called as Protopic, later I found it is a brand and the active constituent is tacrolimus. It is ideal to use the generic name than the brand.

   We appreciate your comment. We changed to generic name.

3) In the in vivo studies there might be some error in quantification of doses, for example, Tacrolimus is used in very high dose 50 mg, in 1% ointment. What is the justification for this dose? The solvent (ethanol) was just 200 microliters.

   We appreciate your comment. Firstly, we used Protopic (consisting 0.1% tacrolimus) as positive control. The dose of 50 mg/mouse is not tacrolimus but Protopic. So, dose of tacrolimus is 0.05 mg/mouse. This dose of tacrolimus is used in previous studies (Lee et al., 2012; Lee et al., 2010).

References


4) Similarly, if the 70% ethanol is used as solvent, the solvent might have interaction with the activity, it might enhance the absorption of the compounds or the extract which is not happening in ideal condition where the ointment bases will not have ethanol.

We appreciate your comment. We have a mistake during preparing manuscript. We used 70% ethanol/PBS (ratio = 1:9) as solvent. This description was corrected in manuscript.

5) Ideally the extract should have made into ointment and its stability should have been assessed and that would have been the ideal proof of concept in animal models.

We appreciate your comment. We agreed with the reviewer’s comment. We were not planned to assess *M. alba* stability in advance. We will be measured the stability of test material in further experiment as suggested by reviewer.

6) The scoring (EASI values) should have been blinded or double blinded during the study. Solely because the investigator might have influence on his positive findings. But again, looking at the histopathological and physical examination it is clear that the extract has significant protection of atopic dermatitis.

We appreciate your comment. We conducted the blind test to measure EASI values. Two veterinarian and three animal study experts participated in the blind test. Histological alternation was examined by two histopathologists.
Reviewer #3:

**Background section**

1) AD is generally used to describe Alzhemier's disorders. It would be better if you could use any other short form to describe atopic dermatitis. If still authors feels that this short form can be used you can proceed with it.

   We appreciate your comment. We corrected “AD” to “atopic dermatitis” in manuscript.

2) Is it a published one or unpublished data. If it is a published data provide reference. If it is unpublished then explain clearly the need of the study (experimental studies on atopic dermatitis).

   We appreciate your comment. There was no study on the effects of *Morus alba* on atopic dermatitis until now. So, we corrected the sentence by commented by reviewer as follows:

   However, there was no study on the effects of *M. alba* on atopic dermatitis.

**Material and Methods section**

1) Any rationale to use hydroalcoholic extract and the percentage yield is also very low.

   We appreciate your comment. *Morus alba* was extracted using 70% ethanol according to previous studies (Nematbakhsh et al., 2013; Hunyadi A et al., 2012)

**References**


2) Why RNS in cell lysate were not measure not measure? (Measurement of NO and PGE₂ production)

We appreciate your comment. We investigated the pharmacological effects including anti-inflammatory, anti-asthma, and anti-cancer effects of herbal materials using various cell lines. Based on these evidence, we applied to many experimental animal model such as atopic dermatitis, asthma, cancer and obesity. During the development of atopic dermatitis, inflammation was an important response. We evaluated NO and PGE₂ as markers to assess the anti-inflammatory effects of Morus alba. As commented by reviewer, RNS is also an important marker. We were not planned to evaluate RNS in advance. We will be measured in further experiment as suggested by reviewer.

3) What you are measuring is nitrite levels not exactly NO levels. Of course nitrite levels are measure of reactive nitrogen species (RNS) including NO. Please appropriate term and later you can relate to inducible NO (Measurement of NO and PGE₂ production).

We appreciate your comment. We agreed with the reviewer’s comment. So, we changed “NO” to “nitrite”

4) You have done exactly as per manufacturer's instruction you have done some modifications. please specify that for manufacturer instruction say's 150 microliter whereas you had used 200 microliter (Induction of atopic dermatitis in NC/Nga mice).

We appreciate your comment. We corrected the sentence commented by reviewer as follows:
as described by the manufacturer with some modifications

5) What is the dose levels tested? kindly specify it (Induction of atopic dermatitis in NC/Nga mice).

We appreciate your comment. In preliminary study, we have used 10 mg/kg and 20 mg/kg of *Morus alba* as experimental doses. The procedure of preliminary study was same to present study. The skin severity significantly decreased in both 10 and 20 mg/kg of *Morus alba* compared with the animal with atopic dermatitis. However, the skin severity of 10 mg/kg more decreased than that of 20 mg/kg. Based on the results of preliminary studies, we determined 10 mg/kg of *Morus alba* as effective dose. These were inserted in Materials and Methods sections.

6) Kindly mention the maximum score that an animal get on your scale (Evaluation of skin severity).

We appreciate your comment. We inserted the sentence as follows:

The range of dermatitis score was 0 to 12

**Statistical analyses**

1) It is unethical to do triplicate on the in-vivo experiments. I do not think the authors would have done it. Kindly correct this statement accordingly.

We appreciate your comment. We inserted the sentence as follows:

All of in vitro experiments were performed at least three times.

2) For non parametric data was is the statistical tool which was used

We appreciate your comment. We inserted the sentence as follows:
The dermatitis score was analyzed using Mann-Whitney’s U test. The concentration of IgE, histamine, PGE₂, nitrite and TARC were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s test. The difference was considered significant at $P < 0.05$.

**Results**

1) It would be better if you share these data so that it will be helpful to know the toxic concentrations of the MAE. If these are already published partly you can provide the reference for it (MAE inhibits nitrite and PGE₂ production in LPS-stimulated RAW264.7 cells).

   **We appreciate your comment. The cytotoxicity of MAE was inserted in Fig. 1.**

2) use this abbreviation along with full text (EASI)

   **We appreciate your comment. We inserted full name (eczema area severity index) for EASI.**

3) This sentence looks like a hyped one write the results only. Do not use words surprise or remarkable, present the results in scientific language rather than marketing language (Surprisingly)

   **We appreciate your comment. We changed “Surprisingly” to “However”**

**Discussion**

1) some of these is reflected in the introduction. few information shared in the introduction need not be discussed here. The information related to the results can be discussed here.

   **We appreciate your comment. We agreed with the reviewer’s comment. First paragraph in Discussion section deleted.**