Author’s response to reviews

Title: A Pilot Single Centre, Double Blind, Placebo Controlled, Randomized, Parallel Study of Calmagen® Dermaceutical Cream and Lotion for the Topical Treatment of Tinea and Onychomycosis

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Version: 1 Date: 01 Jan 2017

Author’s response to reviews:

On behalf of the co-authors of the aforementioned manuscript, we would like to thank the reviewers for their constructive comments. Please find below our reply to their comments and suggestions.

Reviewer #1: The authors present a study of calmagen cream for the treatment of tinea and onychomycosis. Their study is well defined with clear objectives and endpoints. The data is presented clearly and the discussion is relevant. The paper would benefit from photos showing before and after photos for the onychomycosis patients. The discussion should also comment on the consistency of the lotion and cream and how it compares to currently used lacquers for onychomycosis.

Reply: Unfortunately, the photos from the clinical study recovered from the archives of Manipal Acunova, the study coordinator, were not of high resolution for publication. However, since the primary endpoint of the study is mycological cure and the secondary endpoints involving the quantitative measure of size of lesions and severity scores were supported by the presented data in the manuscript, it would be sufficient to overcome the lack of suitable photographs. It is noteworthy that since the completion of this clinical study, more than 300,000 units of Calmagen® have been sold in Australia, New Zealand and Germany with numerous positive testimonials on its efficacy. Nevertheless, the text in page 11, paragraph 2, included a “(data not shown)” clause.
With regards to the consistency of the Calmagen lotion and cream in comparison to currently used lacquers for onychomycosis, we have included a statement below in the Discussion section (page 12, paragraph 5, second sentence).

“Furthermore, Calmagen cream and lotion formulations are based on cosmetic excipients registered with INCI (International Nomenclature of Cosmetic Ingredients), which makes application of Calmagen cream and lotion easier on the skin and nail without any adverse reactions. In contrast, most of the chemical-based anti-fungals have reported skin irritations as a commonly reported adverse event.”

Reviewer #2: This manuscript has been nicely written and relevant to the scope of the science and also contributory to the public.

1. Add figures excluding tables wherever applicable as figure represents data remarkably.

Reply: We have made the following changes:

a. Table 3 (Summary of primary endpoint parameters at baseline vs end of study) has been converted to Figure 2A-C (Calmagen® lotion and cream meet primary endpoint: mycological cure), which further highlights subgroup analysis of the cream (Figure 2B) and lotion (Figure 2C) treatments, which were not presented in the former Table 3. Corresponding text is also included in the discussion (page 9, paragraphs 2-4).

b. Table 5 (Change from baseline and treatment in size (tinea)/surface area involved (onychomycosis)) has been converted to Figure 3A-D (Calmagen® meets secondary endpoints), which includes subgroup analysis of cream (Figures 3A-B) and lotion (Figures C-D). Corresponding text is also included in the discussion (page 10, paragraphs 2-4).

c. A new Figure 4 was added that shows the summary of IGA response. Corresponding text is cited on page 11, paragraph 1).

2. Photographs are not added. Same to be added to increase quality of the manuscript.

Reply: Please refer to reply to Reviewer #1

3. Approval of human ethical committee and approval number is not mentioned in the manuscript.
Reply: The approval from the ethical committee has been mentioned previously in the Declarations (page 14) as Protocol MA-CT-09-12 and approve by the Committee for Evaluation of Protocols for Clinical Research (CLINICOM); Bangalore, India on 22 January 2010. We have included this information in the Methods section (page 6, paragraph 1)

4. Citation of tables in the text at relevant place are not appropriate and even not mentioned at many places. Citation is mandatory.

Reply: We have made the necessary citations. Please also refer to comments on the change of tables into figures.

5. Have you prepared the clinical study protocol in local language? (Part relevant to volunteers)

Reply: The clinical study was explained in the local language orally and both oral and written consent were obtained from volunteer subjects included in the study. The clinical study protocol was in English as it was meant for the PI and study staff who are conversant with English. The Informed consent form (ICF) (having the study information for the patients) provided to the patients was printed in 2 more languages beside English, namely “Hindi” and “Kannada” which were spoken by the subjects.

This has been included in the Methods section, paragraph 1, page 6).

6. What is the status about toxicity or any unwanted reaction after long time exposure of formulations in the nail cavity?

Reply: There has been no reported toxicity or unwanted reaction in the nail cavity even after 6 months of exposure. This was not unexpected since all Calmagen ingredients are registered with INCI (International Nomenclature for Cosmetic Ingredients).

7. Procedure for microbiological study is not dictated anywhere. Necessary to add.

Reply: Please find the detailed protocols below and the corresponding references. However, due to space limitations, the details will not be included in the manuscript, instead the corresponding references will be cited (page 7, paragraph 2) and included in the bibliography and numbered accordingly in the References section (page 17).

Definitive diagnosis of fungal nail infection was made by direct microscopy (a potassium hydroxide KOH preparation) and fungal culture.
Specimens used in the study were hair, nail scrapings and skin scrapings.

**KOH MOUNT:**

The following preparation was done

**SKIN AND HAIR:** 10% Potassium hydroxide was added on the slide to which skin scrapings are added and covered by a glass cover slip and gently preheated before examining for fungi.

**NAIL:** Specimen was placed on a slide, and a drop of 20% KOH was added. A cover slip was applied with gentle pressure to drain away excess KOH. Incubation was done for 2 h or more (up to 48 h) until softening or digestion of the specimen occurred.

**MICROSCOPY/LIVE SPORE COUNT:** Direct microscopic examination of the above specimen was done to detect fungal spores or hyphae. Initial examination was with low power magnification (x10) followed by a higher magnification (x40) for better illumination to study the morphology of the fungus.

**FUNGAL CULTURE**

Digestion of infected-skin materials:

The human skin scale sample weighed 0.0075 g. The entire sample was digested using 1.5 ml of a 0.5% (1:300) trypsin solution plus 1.5 ml of saline - Tween 80 solution. For the first evaluation 0.05 ml to 0.1ml was used. Because of incomplete digestion, a few skin scales could be seen in the fluid as they floated to the top of the pipette. The fluid was distributed with glass spreaders to the surface of the medium.

0.05 ml to 0.1ml of the above suspension was inoculated onto Potato dextrose agar, Malt extract agar and Sabouraud’s Dextrose agar (pH 5.6) containing Cycloheximide and Chloramphenicol at 26°C for 2 to 3 weeks. Mycelium and spores were scraped from the plates and dispersed in a small volume of Sabouraud 2% dextrose broth (usually 20 ml for 25 plates) using a sterile glass homogenizer. After addition of 5% DMSO as a cryoprotectant, the fungal suspension was stored at - 80°C. The viable count was determined by serially diluting the stock in 0.86% NaCl and spreading 50 ul/plate on the Sabouraud’s dextrose agar medium. Colony count was given by the following formula

\[ \text{CFU} = \text{no.of colonies} \times \text{Volume} \times \text{Dilution factor} \]

Plastic phials containing 1.5 ml fungal suspensions with dimethyl sulphoxide at a final conc. of 5% v/v were frozen to -70 deg C at a freezing speed of 1 deg /min. The Vials were then placed in
perforated metal boxes which were stored in 250 l nitrogen containers. Even after an extended period of storage, the fungi exhibited no noticeable loss in viability, resistance to antibiotics or chemotherapeutic agents or pathogenicity.

References:


8. Tables are not drawn properly.

Reply: We have improved Table 1C. We have also re-drawn Table 2. The former Table 4 has been re-drawn and labelled now as Table 3. As earlier mentioned in reply to question#1, Table 3 has been converted to Figure 2 while Table 5 has been converted to Figure 3.

9. Is there any role of regulatory body or authority for conducting experiments on pathogens? Have author taken permission regarding the same?

Reply:

There is a national regulatory body for conducting clinical trials called DCGI (Drug Controller General of India), which oversees trials including ones that involve pathogens.

Since Calmagen is a plant-based topical product (phytopharmaceutical) used as alternative medicine - regulatory permission was not applicable and it was sufficient to conduct studies in an institution with an appropriate ethics committee. The study was conducted at The Apollo Clinic and led by Dr Manoj Parekh, a dermatologist who has extensive medical experience treating patients with tinea and onychomycosis. Ethics committee approval was obtained and the clinical trial is registered in CTRI, The Clinical Trial Registry of India as described in the manuscript CTRI/2012/03/002522.
A new regulatory requirement from the Central Drugs Standards Control Organization (CDSCO) in India came in existence in 2015, which prescribed the regulatory provisions for phytopharmaceuticals. However, the described clinical trial in our manuscript was conducted several years before 2015, therefore was not subject to this new rule.

I hope we have addressed the reviewers’ questions and look forward to having our manuscript accepted for publication at BMC Complementary and Alternative Medicine.

Sincerely,

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