Reviewer's report

**Title:** Topical Application of Concentrated Deep Sea Water (CDSW) reduces inflammation in Atopic Dermatitis-like Skin Lesions in NC/Nga mice

**Version:** 2  **Date:** 17 April 2012

**Reviewer:** Mao-Qiang Man

**Reviewer's report:**

The revised manuscript is improved. There are still rooms, especially English, for further improvement.

1. **Title**
   
   “Application of Concentrated Deep Sea Water Inhibits the Development of Atopic Dermatitis-like Skin Lesions in NC/Nga mice” could be better to reflect the results in this paper.

2. **Abstract** could be revised as (see highlighted words changed by reviewer)

   **Background**

   “Mineral water from deep-sea bedrock, formed over thousands of years, is rich in minerals such as Ca, Mg, Na, K, Fe and others. Our present study was to investigate the preventive effects of natural deep-sea water on developing atopic dermatitis (AD).”

   **Methods**

   We elicited AD by application of DNCB (2,4-dinitro-chlorobenzene) in Nc/Nga mouse dorsal skin. Deep Sea water (DSW) was filtered and concentrated by a nanofiltration process and reverse osmosis. We applied concentrated DSW (CDSW) to lesions five times per week for six weeks, followed by evaluation. 1% pimecrolimus ointment was used as positive control. The severity of skin lesions was assessed macroscopically and histologically. Levels of inflammatory mediators and cytokines in the serum were detected by ELISA and the levels of CD4+ and CD8+ spleen lymphocytes were determined by FACS.

   **Results**

   DNCB-treated mice showed atopic dermatitis-like skin lesions. Treatment of mice with CDSW reduced the severity of symptoms in the skin lesions, including edema, erythema, dryness, and itching. Histological analyses demonstrated that epidermal thickness and infiltration of inflammatory cells were decreased by CDSW treatment. Given these interesting observations, we further evaluated the effect of CDSW on immune responses in this AD model. Treatment of AD mice with CDSW inhibited up-regulation of IgE, histamine, and pro-inflammatory cytokines in the serum. Also, the CD4+/CD8+ ratio in spleen lymphocyte was down-regulated after treatment with CDSW. Finally, cytokines, especially IL-4 and IL-10 which are important for Th2 cell development, were reduced.
Conclusions
Our data suggests that topical application of CDSW could be useful in preventing the development of atopic dermatitis.

3# Introduction
In the last sentence of this section, please replace “treating” with preventing

4# Materials and methods
1). In line 12 of page 6, what is PBS for? In the response to reviewers, the author stated that "we made 2% CDSW with 98% DW".
2). In the last 4th line of page 6, author may know that TEWL is not used to assess dryness/scaling, and Corneometer CM825 measures water content in stratum corneum, not in dermis.

5# Results
1). In line 13 of page 8, please replace “therapeutic” with preventive;
2). Please change “epidermis thickness” to epidermal thickness and do not use “Normal” if it does not appear at the beginning of the sentence;
3). NO data to support the last sentence of the first paragraph on page 9. This sentence could be revised as "These results indicates that CDSW lowers serum histamine and IgE levels in DNCB induced murine AD model"
4). The quality of Figure 2 needs further improvement. Try to use 5 um section.

6# Discussion
1). ON page 12, sentence “Also, to evaluate skin dryness, we measured transepidermal water loss (TEWL) and the moisture content in the dermis” is incorrect. TEWL can not be used to evaluate dryness. Again, moisture content is in stratum corneum not in dermis;
2). In the last second paragraph of page 12, the last sentence could be revised as "These results indicates that CDSW lowers serum histamine and IgE levels in DNCB induced murine AD model"
3). Capital letters should not be used in “Type-1 Hypersensitivity” in the last paragraph of page 12;
4). In the last 9th line of page 13, “regulates” should be changed to “regulate”;
5). On page 13, Sentences “Although total IgE levelswere up-regulated by DNCB, release of cytokines from Th1 cells such as INF-# and IL-2 also increased. Therefore, we believe that production of IgE by B cells was not only stimulated by IL-4, but also by IL-13, or that total IgE levels were increased by IL-4 from cells other than T cells. In addition, increased IFN-# production by CD4+ T cells is related to the chronic nature of AD [38].” Could be deleted.

Conclusion
The last sentence of conclusion could be changed to " Topical application of
CDSW may prove to be an effective approach for preventing other allergic skin diseases.

If the significances are compared to normal control, they should be clearly stated in the figure legends.

See other notes in the manuscript.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

None