

# Anti-inflammatory Potential of *Amauroderma rugosum* Extract in Atopic Dermatitis Models



Ho Ting Shiu<sup>1</sup>, Timothy Man-Yau Cheung<sup>2</sup>, George Pak-Heng Leung<sup>1</sup>

<sup>1</sup>Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China

<sup>2</sup>Tian Ran Healthcare Limited, Hong Kong, China

## Introduction

Atopic dermatitis is a prevalent chronic inflammatory skin disease that significantly impairs patients' quality of life, including intense pruritus, skin irritation, and change in appearance. Current therapeutic approaches, such as corticosteroids and calcineurin inhibitors, often elicit adverse effects like skin thinning and burning sensation upon long-term use. Consequently, patients seek complementary and alternative medicine options, with Traditional Chinese Medicine being a widely embraced approach.

This study investigates the anti-inflammatory potential of the aqueous extract of *Amauroderma rugosum* (AR), an edible mushroom, as a prospective treatment for atopic dermatitis. The polysaccharide (PS) extracted from AR is likely to be the active compound responsible for the observed anti-inflammatory effects.

## Methodology

### Extraction of water extracts and crude polysaccharides from *Amauroderma rugosum*



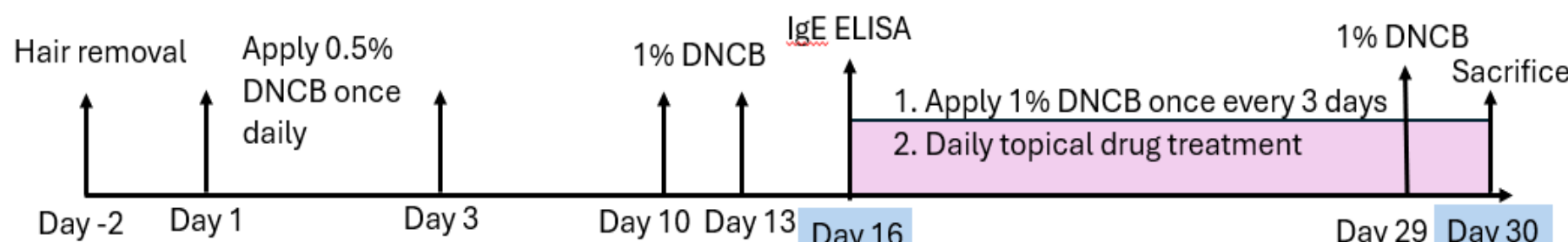
*Amauroderma rugosum* was cultured by Hong Kong Ganoderma Centre Limited (Hong Kong, China). 70 g of AR fruiting bodies were powdered and boiled with 1400 mL distilled water for 1 hour. The extract was collected. The undissolved residue was continued to boil in water twice. After that, all the extractions were pooled and condensed by rotary evaporator. The condensed extract were subjected to freeze dry to a power form and store at -80°C until further use. To prepare the crude polysaccharide, the condensed AR extraction was mixed with 85% ethanol at a ratio of 1:4 for overnight precipitation at 4°C. Finally, the precipitation was collected by centrifugation and freeze-dried to obtain the crude polysaccharide powder. The polysaccharide content was quantified using Total Carbohydrate Assay Kit purchased from Abcam.

### Cell Culture and Drug Treatment

Inflammation of HaCaT human keratinocytes was induced by 10 ng/mL human recombinant tumor necrosis factor (TNF)-α and 10 ng/mL human recombinant interferon (IFN)-γ for 24 h. The cells were co-treated with or without different concentrations of drug extracts.

### Animal model

To establish the atopic dermatitis model, 8-10 weeks old BALB/c mice were sensitized with 0.5% 2,4-dinitrochlorobenzene (DNCB) solution dissolved in acetone/olive oil (3:1) for 3 days, followed by repeated challenge of 1% DNCB every 3 days until day 30. Topical application of drug treatment commenced on day 16.



## Conclusion

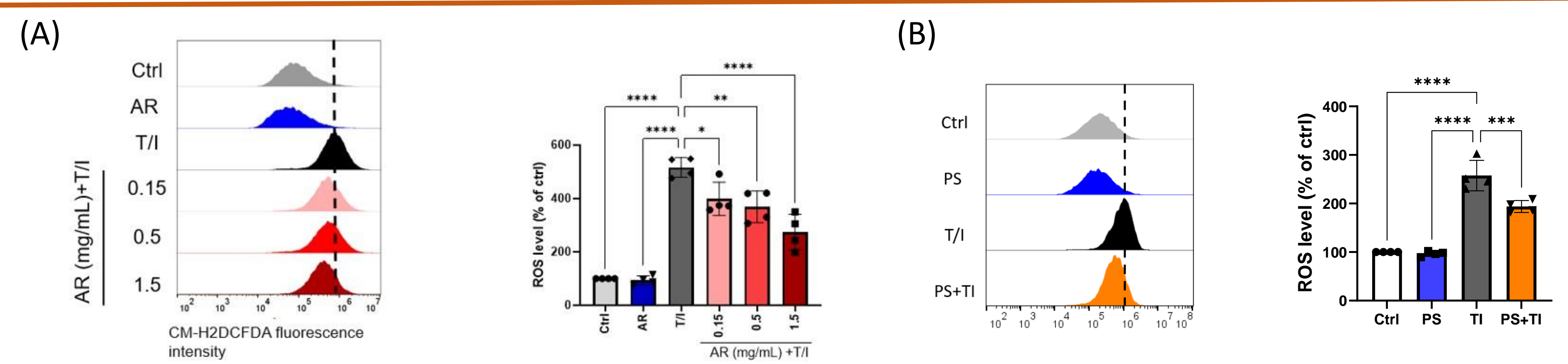
This study demonstrated the potent anti-inflammatory effects of the aqueous extract and polysaccharides from the edible mushroom *Amauroderma rugosum* (AR) in both in vitro and in vivo models of atopic dermatitis. The AR water extract and isolated polysaccharides effectively suppressed the production of inflammatory mediators in human keratinocytes. Moreover, topical application of the AR polysaccharides significantly ameliorated disease symptoms and reduced the levels of these pro-inflammatory factors in the dorsal skin tissue of a mouse model of atopic dermatitis. Future studies will focus on identifying the optimal drug delivery system to enhance the topical application and improve the anti-inflammatory efficacy of the AR polysaccharides.

## Acknowledgement

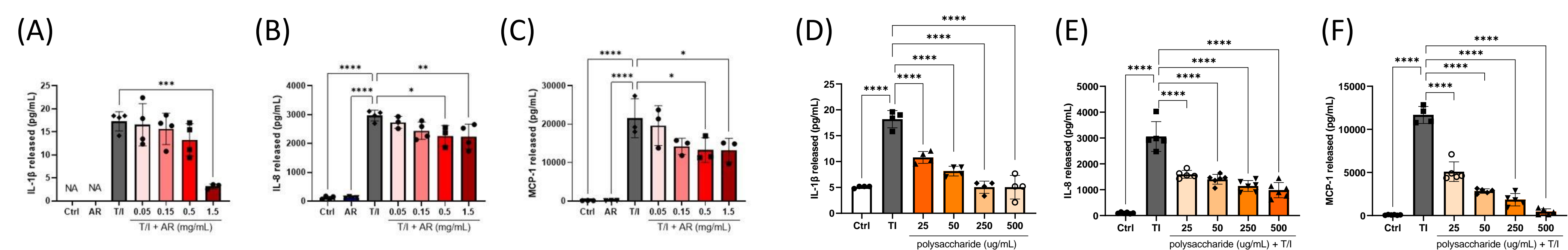
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## Results

### Result 1: AR Water Extract and its Polysaccharide Suppress Oxidative Stress and Inflammatory Markers in an Inflamed Keratinocyte Cell Model

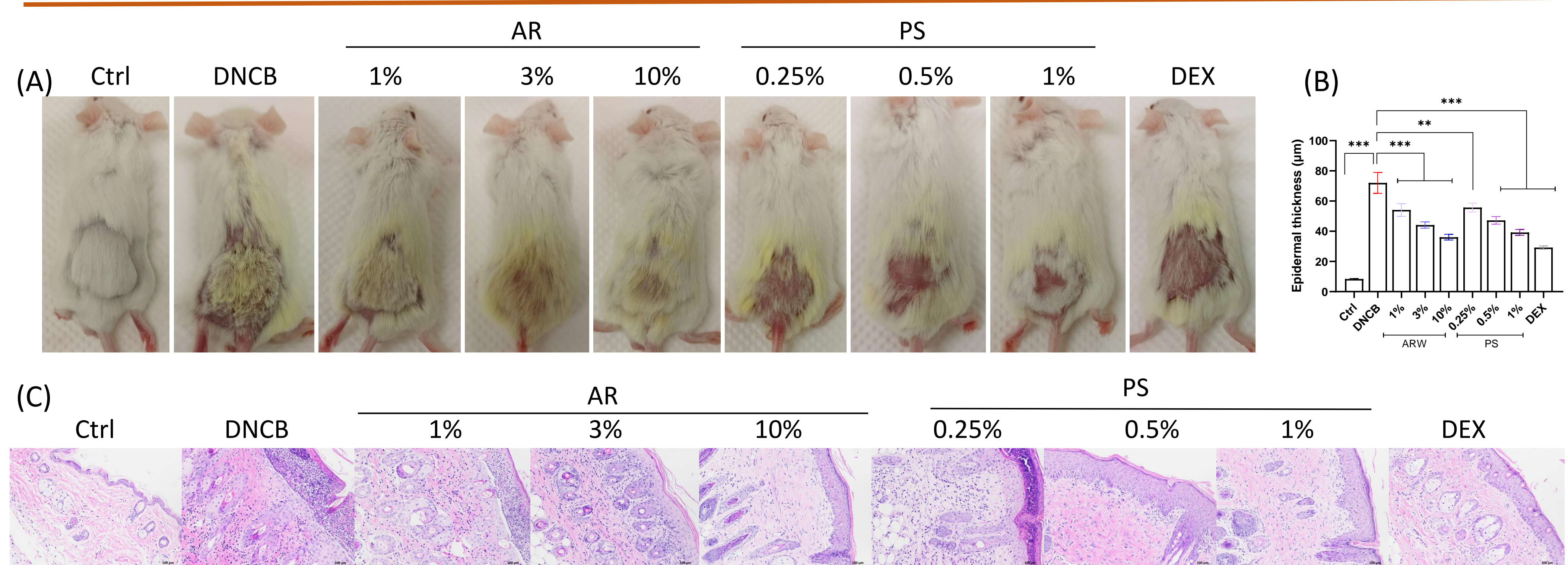


**Figure 1.** AR water extract (A) or crude polysaccharide (B) reduced the production of reactive oxygen species (ROS) induced by TNF-α and IFN-γ in HaCaT cells. Intracellular ROS levels were quantified by flow cytometry after staining the cells with the fluorescent dye CM-H2DCFDA.

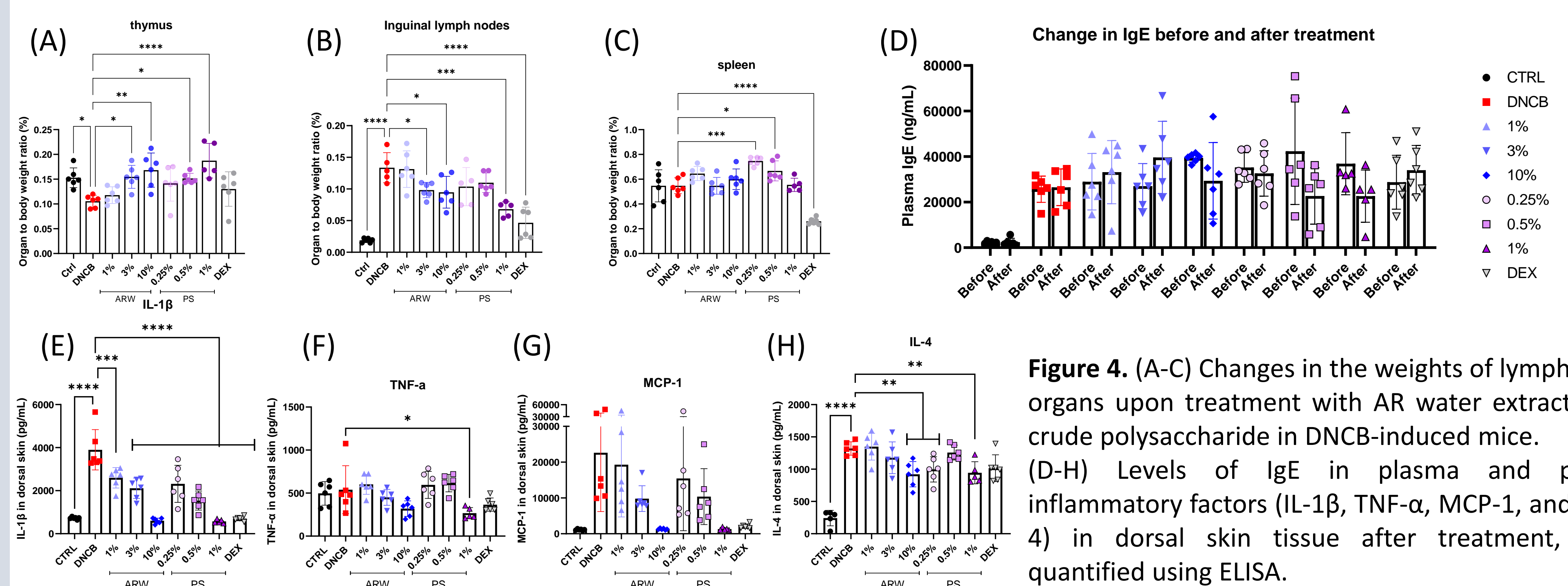


**Figure 2.** AR water extract (A, B, C) or crude polysaccharide (D, E, F) reduced the release of pro-inflammatory cytokines and chemokines (IL-1β, IL-8 and MCP-1) from TNF-α/IFN-γ-induced HaCaT cells.

### Result 2: AR Water Extract and its Polysaccharide Ameliorates Clinical Symptoms and Modulates Immunological Markers in DNCB-induced mice



**Figure 3.** (A) Representative images of the dorsal skin in DNCB-induced mice after treatment with AR water extract or crude polysaccharide. (B) Effects of AR water extract and crude polysaccharide treatment on the epidermal thickness of the dorsal skin. (C) Representative images of dorsal skin stained with H&E to quantify the epidermal thickness.



**Figure 4.** (A-C) Changes in the weights of lymphoid organs upon treatment with AR water extract or crude polysaccharide in DNCB-induced mice. (D-H) Levels of IgE in plasma and pro-inflammatory factors (IL-1β, TNF-α, MCP-1, and IL-4) in dorsal skin tissue after treatment, as quantified using ELISA.