

RESEARCH ARTICLE

In vitro skin permeation and anti-atopic efficacy of lipid nanocarriers containing water soluble extracts of *Houttuynia cordata*

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Abstract

Objective: The aims of this work are to enhance the *in vitro* skin permeation of *Houttuynia cordata* (water-soluble extract of *H. cordata*; HCWSE) and to boost the efficacy of HCWSE against atopic dermatitis (AD) – like skin lesion in hairless mice using lipid nano-carriers (liposome and cubosome).

Methods: HCWSE was obtained by a hot water extraction. Monoolein cubosomal suspension containing HCWSE and egg phosphatidylcholine liposomal suspension containing the same was prepared by a sonication and a film hydration method, respectively.

Results: The lipid nano-carriers, especially cubosome, enhanced the *in vitro* skin permeation of HCWSE. The inhibitory effects of HCWSE-containing lipid carrier suspensions on the development of 1-chloro-2,4-dinitrobenzene (DNCB)-induced AD-like skin lesion in hairless mice were investigated by observing appearance of skin surface, serum immunoglobulin E (IgE) level and cytokine expression. HCWSE-containing preparations suppressed IgE production and interleukin 4 expression, whereas they promoted interferon gamma expression. The order of lymphocyte (B-cell, Th1 cell and Th2 cell) modulating effect was HCWSE-containing cubosomal suspension > HCWSE-containing liposomal suspension > HCWSE solution in phosphate buffered saline, indicating that the cubosomal suspension, among the preparations, was the most efficacious in inhibiting the development of DNCB-induced AD-like skin lesion.

Conclusion: It is believed that the cubosomal suspension containing HCWSE would be an efficacious preparation for the treatment of AD.

Keywords

Atopic dermatitis, cubosome, cytokines, *Houttuynia cordata*, immunoglobulin E, liposome

History

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder and patient suffering from AD have been increasing in number. Abnormal immunological response and skin barrier malfunction are known to be main pathogenesis¹. Common immunological characteristic of AD is that Th2 cell response overwhelm Th1 cell response^{2,3}. As a result of the immunological abnormality, immunoglobulin E (IgE) is overproduced, Th2 cytokines (e.g. interleukin 4; IL-4) are over-expressed and Th1 cytokines (e.g. interferon gamma; IFN- γ) are under-expressed^{4,5}.

Lipid nano-carriers such as liposomes and cubosomes have been proposed as carriers for skin disease, because they are mainly composed of non-toxic lipids (e.g. phospholipid and monoolein; MO) and enhance the skin permeations of bioactive ingredients⁶. Liposome is phospholipid bilayer vesicles, and it is known to be an efficacious dermal delivery carrier for acne⁷, psoriasis⁸, eczema⁹ and AD¹⁰. Cubosome is nanoparticle of cubic phase, and it is usually prepared using MO. Inter-crossing water channels go through the lipid matrix of nanoparticles. Cubosome was also proposed as an effective dermal delivery carrier for various types of skin diseases¹¹. The common features of liposome and cubosome are that they are composed of biocompatible lipid, nano-sized and that they can accommodate

not only oil-soluble compounds but also water-soluble compounds. Due to their features as drug carriers, the thermodynamic activity of water soluble compound increases upon being loaded in the lipid carriers so the skin permeation is enhanced^{12,13}.

Houttuynia cordata (HC) is a medicinal herb for Herpes simplex, nasal polyps and chronic sinusitis, and its water extract was claimed for anti-leukemic activity, anti-oxidation activity¹⁴ and anti-cancer activity¹⁵. Recently, the extract is reported to be efficacious in treating AD¹⁶. The skin permeation of the water soluble extract is required to be promoted for the enhancement of the bioavailability and the efficacy of the extract. Since water-soluble compound has a low thermodynamic activity in an aqueous preparation, the permeation through skin is usually poor¹⁷.

The aim of this work is to overcome the low skin permeation and to enhance the anti-AD efficacy of water-soluble extract of HC (HCWSE). MO cubosomal suspension and egg phosphatidylcholine (EPC) liposomal suspension, each containing HCWSE, were prepared by a film hydration method. The *in vitro* skin permeations of HCWSE contained in the cubosomal suspension and in the liposomal suspension, and of HCWSE dissolved in phosphate-buffered saline were investigated using hairless mouse skin mounted on a Franz-diffusion cell. Furthermore, AD-like skin lesion was induced in hairless mice using 1-chloro-2,4-dinitrobenzene (DNCB), and the inhibitory effects of HCWSE-containing preparations on the development of AD-like skin lesion were investigated by observing appearance of skin surface microscopically, serum IgE level and cytokine expression.

Materials and methods

Materials

MO (1-monooleoyl glycerol, MO) was gifted by Danisco Ingredients A/S (Copenhagen, Denmark) (monoglyceride content is approx. 95.7% and oleic acid content is approx. 90%). EPC, DNCB, Pluronic F127, phosphotungstic acid and phosphoric acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). Phosphate buffered saline (PBS) was purchased from Bio-Rad Laboratories, Inc. (Berkeley, CA). Methyl alcohol was provided by Dae Jung Co. (Siheung, Korea). HC was supplied by DermaLab (Wonju, Korea). Water was distilled in a water purification system (Pure Power I⁺, Human Corporation, Seoul, Korea) until the resistivity was 18 M Ω /cm. All other reagents were in analytical grade.

Animals

Six-week-old female hairless mice were provided by Orient Bio (Sungnam, Korea). The animals were kept in a constant temperature (22 $\pm$ 2 $^{\circ}$ C) and humidity (55 $\pm$ 5%) environment and fed with a standard diet and water throughout the experiment.

Extraction of water-soluble extract from HC

Fifty grams of dry leaves was put into 1L of distilled water contained in a three-neck round bottom flask with a vertical cooling condenser. The extraction was done at 100 $^{\circ}$ C for 12 h. Then, the mixture was allowed to cool to room temperature, filtered, thickened and freeze-dried. The yield of extraction (%) was calculated by the equation of (mass of dry extract)/(mass of dry leaves) $\times$ 100.

Preparation of liposomal suspension containing HCWSE

The liposomal suspension was prepared by a dry film hydration method^{18–20}. Then 0.2 ml of EPC solution in chloroform (100 mg/ml) was put in a 25 ml round bottom flask. The dry thin film of EPC was obtained by evaporating organic solvent in a rotary evaporator operating at 45 rpm and 37 $^{\circ}$ C. The dry lipid film was hydrated with 2 ml of HCWSE solution (2%) in PBS (pH 7.4) by swirling the flask at room temperature until lipid film was completely detached from the wall of round bottom flask. Then, the lipid suspension was homogenized in a bath type sonicator (VC 505, Sonic & Materials, Newtown, CT, 30% energy intensity, 30 s pulse on, 30 s pulse off) at room temperature for 15 min.

Preparations of cubosomal suspension containing HCWSE

Cubosomal suspension was prepared by a method described elsewhere²¹. Two grams of MO were melted in a water bath, kept at 60 $^{\circ}$ C, and 0.879 g of HCWSE solution (2%) in distilled water was layered over the molten MO, so the content of water is 30%. The two-phase systems were kept at 25 $^{\circ}$ C until clear gels were obtained. To obtain the nanoparticles of cubic phase, 200 ml of Pluronic F127 solution in the PBS (pH 7.4) buffer (1.5 mg/ml) was added to the MO gel so that the ratio of the polymeric surfactant to MO was 15:100. Then, the gels were micronized in a bath type sonicator (VC 505, Sonic & Materials, 30% energy intensity, 30 s pulse on, 30 s pulse off) at room temperature for 15 min.

Transmission electron microscopy of lipid nanocarriers

Liposome and cubosome were negatively stained with freshly prepared phosphotungstic acid solution (2%, pH 6.8)²². By soaking formvar/carbon coated grids (200 mesh) in stained liposomal and cubosomal suspensions, the lipid particles were

transferred onto the grid. The transmission electron microscopy (TEM) photos were taken on an electron microscope (LEO-912AB OMEGA, LEO, Waldbronn, Germany).

Size measurements

The lipid contents in liposomal and cubosomal suspensions were adjusted to 0.03% by diluting the suspensions using PBS (pH 7.4). The mean diameters and size distributions were determined using a particle size analyzer (ZetaPlus 90, Brookhaven Instrument Co., Holtsville, NY).

In vitro skin permeation

In vitro skin permeations of HCWSE contained in liposomal and cubosomal suspensions were observed by a method reported elsewhere²³. The dorsal skins of 6-week-old female hairless mice (type SKH 1) were mounted on a Franz diffusion cells (Lab Fine Instruments, Seoul, Korea). PBS (pH 7.4) was used as a receptor solution and it was kept at 37 $^{\circ}$ C under stirring throughout the experiment. Then 200 μ l of liposomal suspension and cubosomal suspension, of which HCWSE concentrations were 2%, were applied on skin (0.636 cm²), and 100 μ l of receptor solution was taken for the determination of HCWSE concentration at a given time. HCWSE solutions (2.0%) in PBS (pH 7.4) was used as controls. HCWSE was assayed using a HPLC (M600E, M7725i/Waters, 996PDA) equipped with a UV detector (0.05 AFUS) (located at Central Laboratory of Kangwon National University). A reversed phase column (4.6 $\times$ 250 mm, X Bridge C18 5 μ m) was eluted with 0.5% phosphoric acid/methanol (1/1, v/v) at a flow rate of 1.0 ml/min, and a sample of 50 μ l was injected. The detection wave length was 349 nm.

Induction of AD-like skin lesions in hairless mice

One hundred microliters of DNCB solution (1%) in acetone was daily applied for 1 week on backs of mice in control groups and experimental groups. And then, 100 μ l of DNCB solution (0.5%) in acetone was applied every two days for 5 weeks. In parallel, 100 μ l of each of PBS (pH 7.4) alone, HCWSE contained in cubosomal suspension, HCWSE contained in liposomal suspension and HCWSE dissolved in PBS (pH 7.4) was daily applied on the backs of mice in experimental groups for 5 weeks. AD-like skin lesions were investigated at the end of every week for 6 weeks by a skin diagnosis system (Aramo TS, Aram HUVIS Co., Ltd. Sungnam, Korea). At the end of experiment, immunological evaluations were done by determining the concentration of IgE and cytokines in serum.

Determination of IgE concentration

At the end of the 6-week experiment for the evaluation of anti-atopic activity, blood was collected from the eye and heart using a heparinized capillary tube and injector, and it was centrifuged at 5000 rpm, 4 $^{\circ}$ C for 10 min to separate serum. The concentration of IgE in serum was determined by immunoperoxidase assay kit (Gen Way Biotech, Inc. San Diego, CA). Five microliters of serum was diluted with 45 μ l of buffer, and the diluted serum was dispensed in each well of 96-well plates. The plate was gently shaken on a shaker (VWR 1535 Incubator Shaker 96well Plate Shaker, Triad Scientific, Manassquan, NJ) at room temperature for 2 h, and then it was washed with buffer solution three times, each time for 5 min. Then, biotin-conjugated anti-IgE antibody was put in the wells, and it was shaken on a shaker at room temperature for 2 h. After the plate was washed with buffer solution three times, 50 μ l of HRP-conjugated avidin was put in the well, and it was shaken on a shaker at room temperature for 2 h. After the plate was washed with buffer solution three times, 50 μ l of

chromogenic substrate (TMB) reagent was put in the well, it was kept at room temperature for 5 min, 50 μ l of stop solution was put to the well, and finally the absorbance of the reagent in each well was determined by ELISA reader (450 nm).

Determination of cytokine concentration

The concentrations of IL-4 and IFN- γ in serum were determined using an enzyme immunometric assay kit (Assay Designs, Inc., Ann Arbor, MI). One hundred microliters of capture antibody, which had been 180 times diluted with PBS, was dispensed to each well of 96-well plate, and it was kept at room temperature overnight. Then, 300 μ l of block buffer was dispensed to each well containing capture antibody, and the reaction was done at room temperature for 1 h. Then, samples and standard were diluted with reagent diluents, they, 100 μ l of each, were dispensed to the wells containing capture antibody, and the reaction was done at room temperature for 2 h. Streptavidin–HRP was diluted with working diluents, 100 μ l of diluted streptavidin–HRP was dispensed to the wells, and the reaction was done at room temperature for 20 min. In subsequent, 100 μ l of substrate solution was dispensed to the wells, and the reaction was done at room temperature for 20 min. The plate was washed with PBS-Tween (0.05%) in each step. After a color was developed in each well, the reaction was stopped by dispensing 50 μ l of 2 N H₂SO₄ to each well. The absorbance was determined by ELISA reader (450 nm). A calibration curve was set up using purified cytokine.

Statistical analysis

The statistical differences between normal group versus control group and experimental groups, and control group versus experimental groups were analyzed with Student's *t*-tests. All data are shown as mean $\pm$ SEM ($n = 4$).

Results and discussion

Extraction of water-soluble extract from HC

The yield of extraction was 24.7%. The retention time of one of the major peaks of HCWSE on HPLC was 6.76 min. The equation of calibration curve for HCWSE was $Y = 12946.8 X + 47.2$ ($R^2 = 0.9998$), where *X* is the concentration of HCWSE in PBS (10 mM, pH 7.4) in μ g/ml and *Y* is the area of peak at the retention time. The limit of detection and the limit of quantification were 0.27 μ g/ml and 0.81 μ g/ml, respectively.

Preparation of liposomal suspension containing HCWSE

Figure 1 shows the size distribution of liposomes with and without HCWSE. The distributions were bimodal whether HCWSE was included or not. In the distribution of liposomes with HCWSE (Figure 1A), the first population ranged from 100 to 130 nm and the second population ranged from 330 to 470 nm. The mean diameter was 273.3 nm. In the distribution of liposomes without HCWSE (Figure 1B), the first population ranged from 90 to 95 nm and the second population ranged from 154 to 340 nm. And the mean diameter was 242.4 nm. The inhomogeneity in size is possibly due to mild sonication during the homogenizing process. More intensive sonication would lead to a monomodal distribution but could deteriorate HCWSE and phospholipid. There was no significant difference in the size distribution and the mean diameter, between liposomes with and without HCWSE. A compound could affect the size and the structure of liposomes by hydrophobic interaction if it is oil-soluble and surface-active. In addition, an electrostatic interaction between a compound and liposomal membrane has an effect on the size and the structure. Since HCWSE is water-soluble and egg

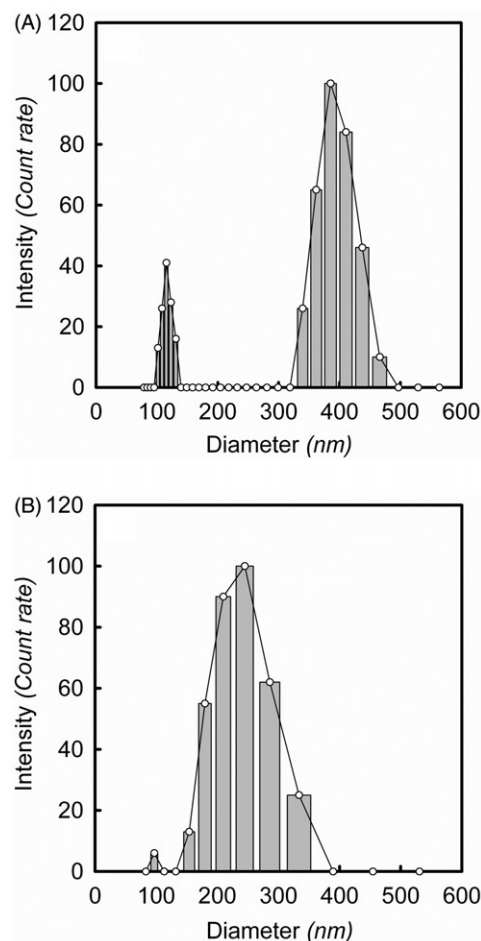


Figure 1. Size distributions of liposomes with (A) and without (B) HCWSE.

PC is electrically neutral, there would be no hydrophobic and electrostatic interaction between HCWSE and the liposomal membrane. This could account for why no big difference in the mean size and the size distribution was observed.

Figure 2 shows the TEM photos of liposomes with and without HCWSE. Multilamellar closed vesicles were observed, and the size ranged from 100 to 300 nm. There was no big difference in the structure and the size between liposomes with and without HCWSE. HCWSE would hardly interact with liposomal membrane because it is a water-soluble compound and the liposomal membrane is electrically neutral.

Preparation of cubosomal suspension containing HCWSE

Figure 3 shows the size distribution of cubosomes with and without HCWSE. The distributions were bimodal whether HCWSE was included or not. In the distribution of cubosomes with HCWSE (Figure 3A), smaller population ranged from 73 to 90 nm and larger population ranged from 216 to 300 nm. The mean diameter was 231.7 nm. In the distribution of cubosomes without HCWSE (Figure 3B), smaller population ranged from 35 to 50 nm and larger population ranged from 215 to 335 nm. And the mean diameter was 235.4 nm. Figure 4 shows the TEM photos of cubosomes with and without HCWSE. The particles were striped and the size ranged from 80 to 150 nm. During the staining process, phosphotungstic acid, a heavy metal, will penetrate into water channels of cubosomes. Black-and-white stripes on the particles can be ascribed to the particle structures that water channels go through lipid matrix. There was no big difference in size and structure between cubosomes with and without HCWSE.

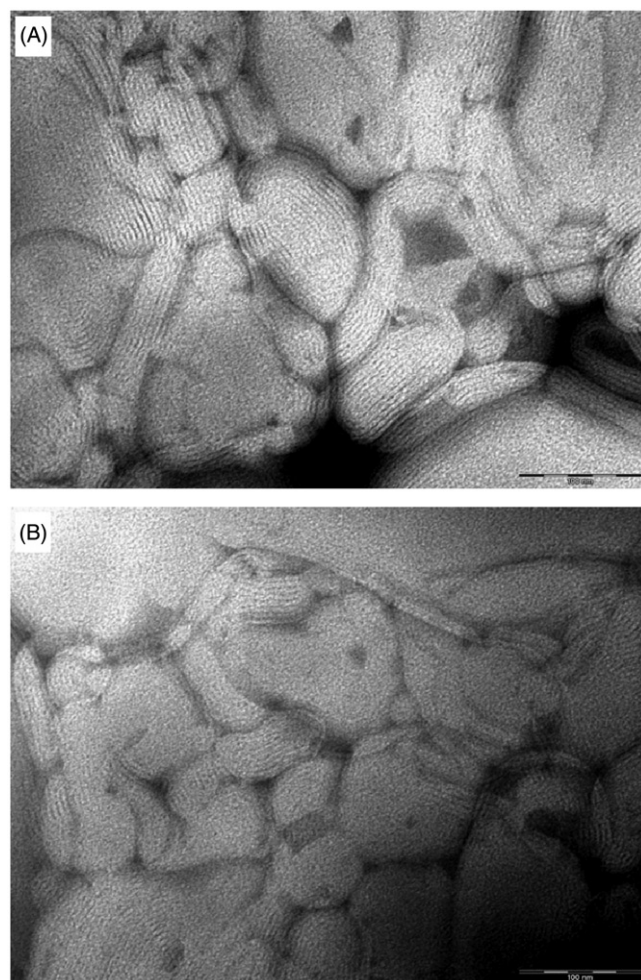


Figure 2. TEM photos of liposomes with (A) and without (B) HCWSE. The liposomes were negatively stained with phosphotungstic acid solution (2%, pH 6.8). Bar in each photo represents 100 nm.

Since HCWSE is water-soluble and MO is electrically neutral, there will be no hydrophobic and electrostatic interactions between HCWSE and MO cubosomal membranes. This would account for why HCWSE had no great effect on the size and the structure of cubosomes.

Differential scanning calorimetry of cubic phase

Figure 5 shows the thermogram of MO, cubic phase with and without HCWSE. The endothermic peak of MO was observed around 33 °C and it is due to the melting of MO. The endothermic peak of cubic phase was observed around 68 °C and it is due to the phase transition to hexagonal phase²⁴. The endothermic peak of cubic phase containing HCWSE was observed at almost the same temperature where the phase transition of cubic phase containing no HCWSE occurred. It means that HCWSE has little effect on the structure of cubic phase. As described previously, there would no significant interaction between HCWSE and MO cubic phase, because HCWSE is water-soluble and MO is electrically neutral.

In vitro skin permeation

Figure 6 shows the skin permeation of HCWSE contained in cubosomal suspension, HCWSE contained in liposomal suspension and HCWSE dissolved in PBS (pH 7.4). The accumulative amount of HCWSE in a receptor cell was in the order of HCWSE in cubosomal suspension > HCWSE in liposomal suspension > HCWSE in PBS. For example, when HCWSE was contained in

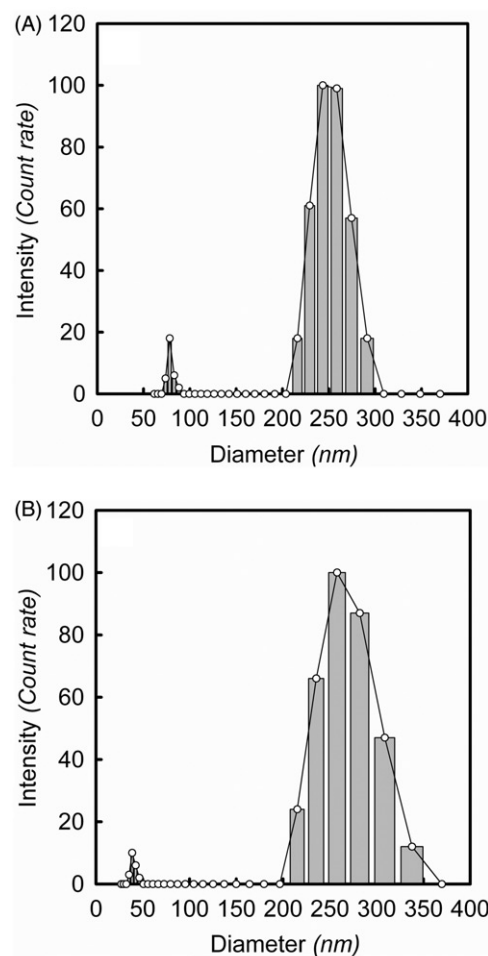


Figure 3. Size distributions of cubosomes with (A) and without (B) HCWSE.

cubosomal suspension, liposomal suspension and PBS, the amount in 24 h was 11.53 $\mu\text{g}/\text{cm}^2$, 8.49 $\mu\text{g}/\text{cm}^2$ and 6.85 $\mu\text{g}/\text{cm}^2$, respectively. Lipids such as phospholipid (e.g. EPC) and glyceride (e.g. MO) are known to interact with skin, and act as skin permeation enhancers^{25,26}. Moreover, liposome and cubosome could penetrate into skin through paracellular routes^{27,28}. The mean size of cubosomes was smaller than the mean size of liposomes. The smaller size of cubosome could explain the higher skin permeation of HCWSE in cubosomal suspension. On the other hand, the skin permeation of HCWSE dissolved in PBS was much lower than that of HCWSE in the lipid carrier suspensions. Because HCWSE is water-soluble, it would have a low thermodynamic activity in PBS. This would be a reason why the skin permeation of HCWSE in PBS is low.

Appearance of skin surface

Figure 7 shows the effects of HCWSE contained in cubosomal suspension, liposomal suspension and PBS on the appearance of dorsal skin surface of mouse having DNCB-induced AD-like skin lesion. Rugged and bumpy skin was found with mouse in control group throughout experiment (Figure 7B). The rough skin is known to be characteristic of DNCB-induced AD-like skin lesion⁴. Compared with the control, PBS (pH 7.4) alone seems to have a little suppressing effect on AD-like skin lesion (Figure 7C). Mouse suffering from AD-like skin lesion has a low skin barrier function, so the epidermal water loss of atopic mouse is high and the skin is in dry state^{29,30}. The application of PBS (pH 7.4) alone might somewhat compensate for the water loss, leading

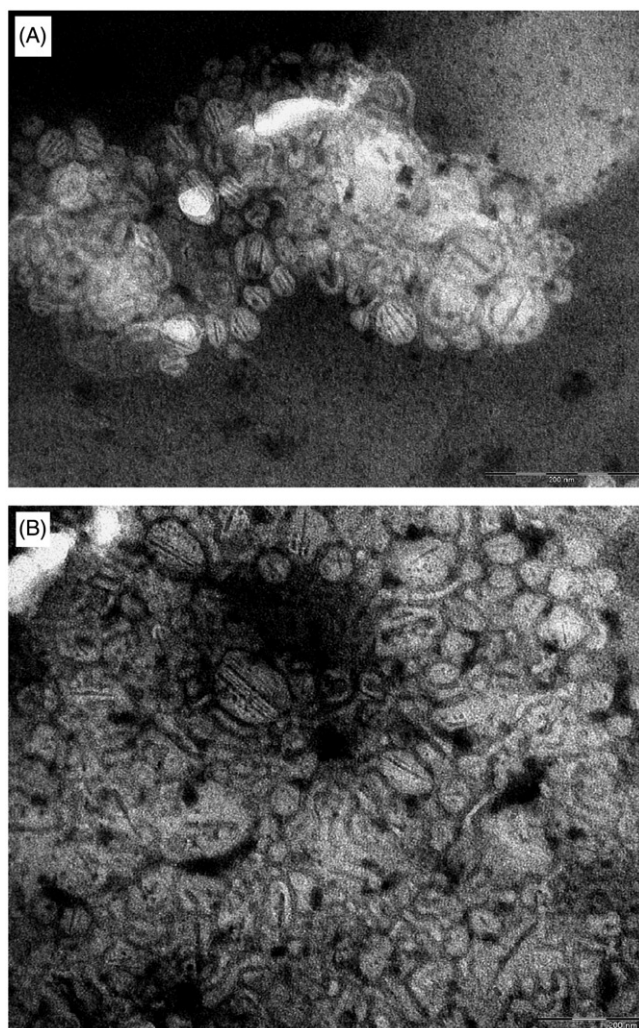


Figure 4. TEM photos of cubosomes with (A) and without (B) HCWSE. The cubosomes were negatively stained with phosphotungstic acid solution (2%, pH 6.8). Bar in each photo represents 200 nm.

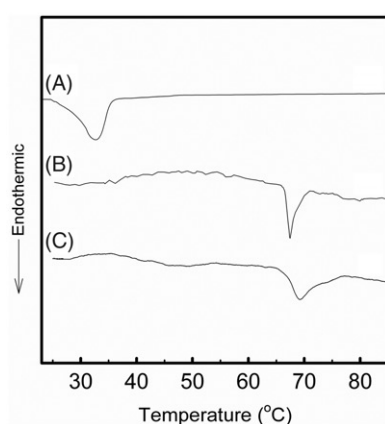


Figure 5. Thermograms of MO (A) and cubic phases with (B) and without (C) HCWSE.

to suppression of the lesion. HCWSE contained in cubosomal suspension had a significant inhibitory effect on the development of AD-like skin lesion. Four weeks after the cubosomal suspension was applied, the rugged and rough skin became smooth. Six weeks after the application, the skin surface was apparently almost the same as the skin surface of normal mouse.

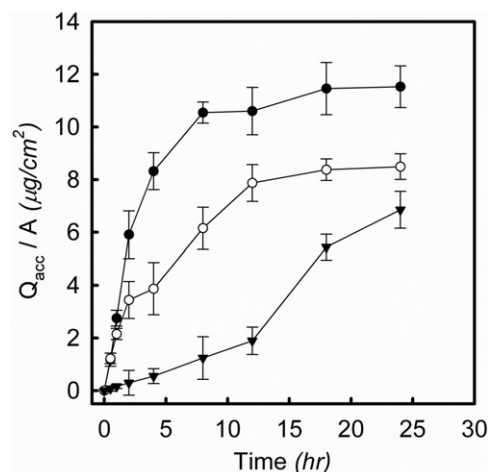


Figure 6. *In vitro* skin permeation of HCWSE contained in cubosomal suspension (●), HCWSE contained in liposomal suspension (○) and HCWSE dissolved in PBS (▼). Q_{acc} is accumulated amount of HCWSE in receptor solution, and A is skin area exposed to HCWSE preparations (0.636 cm^2).

According to the result of skin permeation (Figure 6), cubosomes effectively enhanced the skin permeation of HCWSE so it could boost the anti-atopic efficacy of HCWSE. In addition, cubosomes are mainly composed of MO so it could reinforce the skin barrier function by its retention in skin. These would be reasons why HCWSE contained in the cubosomal suspension had a significant inhibitory effect on the development of AD-like skin lesion. HCWSE contained in liposomal suspension also exhibited a high efficacy in term of smoothing AD-like skin (Figure 7E), but seemed to be less efficacious than HCWSE contained in cubosomal suspension. The skin permeation-enhancing effect of liposome was less than that of cubosome, and it may account for why the skin-smoothing effect of HCWSE contained in liposomal suspension was somewhat less.

IgE level

Figure 8 shows serum IgE level in normal, control and experimental groups. When hairless mice were treated with DNCB, IgE level increased up to $456.45 \pm 28.80 \text{ ng/ml}$. A marked increase is possibly because the expression of Th1/Th2 cytokines in the spleen was unbalanced by DNCB^{2–5}. In fact, serum IgE level is known to increase in patients suffering from AD and hairless mice with AD-like skin lesions^{1,31}. Thus, serum IgE level can be used as a measurement to assess the capability of HCWSE preparations to modulate DNCB-induced immune response. Serum IgE level decreased by the application of preparations containing HCWSE. The degree of reduction in IgE production was in the order of HCWSE contained in cubosomal suspension > HCWSE contained in liposomal suspension > HCWSE dissolved in PBS. It means that, among the preparations tested, HCWSE contained in cubosomal suspension had the greatest inhibitory effect on the development of AD-like skin lesion.

IL-4 expression

Figure 9 shows IL-4 expression in mice with AD-like skin lesion. When the hairless mouse was treated with DNCB, the amount of IL-4 markedly increased. Since IL-4 (Th2 cytokines) is reported to play a key role in the overproduction of IgE, IL-4 expression, together with IgE level, can be used as a measurement to assess the capability of HCWSE preparations to modulate DNCB-induced immune response. IL-4 expression was markedly decreased by the application of preparations containing

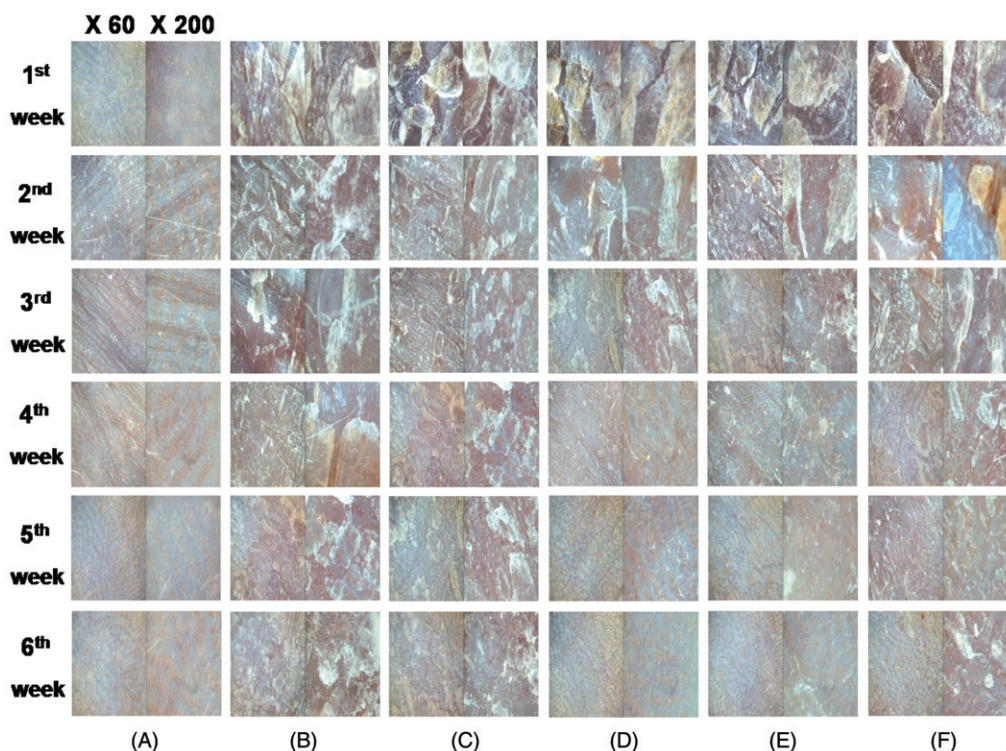


Figure 7. Appearance of dorsal skin surface of normal mouse (A) and mice suffering from DNCB-induced AD-like skin lesion (B–F), which were treated with nothing (B), PBS alone (C), HCWSE contained in cubosomal suspension (D), HCWSE contained in liposomal suspension (E) and HCWSE dissolved in PBS (F). Magnification of left and right side in each column was 60 and 200 times, respectively.

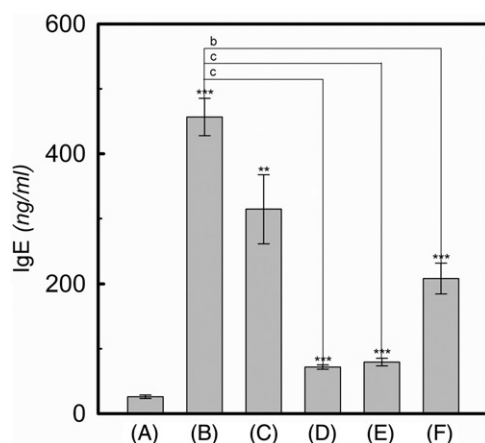


Figure 8. Serum IgE level in normal mouse (A) and mice suffering from DNCB-induced AD-like skin lesion (B–F), which were treated with nothing (B), PBS alone (C), HCWSE contained in cubosomal suspension (D), HCWSE contained in liposomal suspension (E) and HCWSE dissolved in PBS (F). Values are means $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.001$.

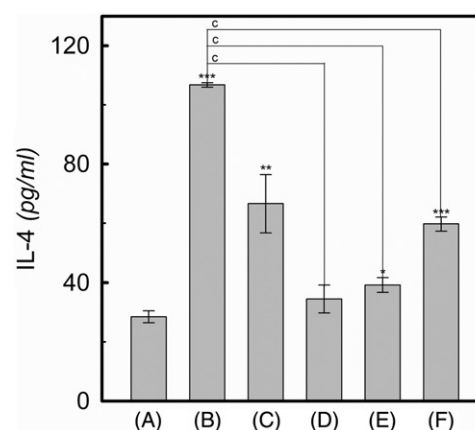


Figure 9. IL-4 expression in normal mouse (A) and mice suffering from DNCB-induced AD-like skin lesion (B–F), which were treated with nothing (B), PBS alone (C), HCWSE contained in cubosomal suspension (D), HCWSE contained in liposomal suspension (E) and HCWSE dissolved in PBS (F). Values are means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^c $p < 0.001$.

HCWSE. The degree of reduction in IL-4 expression was in the order of HCWSE contained in cubosomal suspension > HCWSE contained in liposomal suspension > HCWSE dissolved in PBS. The order of IL-4 expression extent was the same as that of IgE level. This is possibly because the production of IgE is proportional to the degree of IL-4 expression⁴.

IFN- γ expression

Figure 10 shows IFN- γ expression in mice with AD-like skin lesion. When the hairless mouse was treated with DNCB, the amount of IFN- γ decreased. IFN- γ (Th1 cytokine) is known to suppress the synthesis of IgE by inhibiting Th2 cell proliferation and IL-4 receptor expression on T-cells³². IFN- γ expressions

increased upon the application of HCWSE-containing preparations. The degree of increase in IFN- γ expression was in the order of HCWSE contained in cubosomal suspension > HCWSE contained in liposomal suspension > HCWSE dissolved in PBS. The order of IFN- γ expression extent was inverse to the order of IgE level and that of IL-4 expression extent. The inverse order is possibly because IL-4 (Th2 cytokines) promotes the production of IgE, and IFN- γ (Th1 cytokines) inhibits IgE synthesis³³.

Following the results of IgE production, IL-4 expression and IFN- γ expression, it is concluded that HCWSE contained in cubosomal suspension, among the preparations tested, had the greatest inhibitory effect on the development of AD-like skin lesion. Cubosome was the most effective in enhancing the skin permeation of HCWSE (Figure 6), and this may be one of the

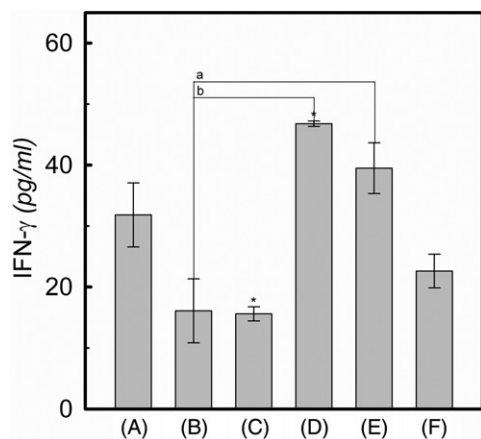


Figure 10. IFN- γ expression in normal mouse (A) and mice suffering from DNCB-induced AD-like skin lesion (B–F), which were treated with nothing (B), PBS alone (C), HCWSE contained in cubosomal suspension (D), HCWSE contained in liposomal suspension (E) and HCWSE dissolved in PBS (F). Values are means $\pm$ SEM. * $p < 0.05$, ^a $p < 0.05$, ^b $p < 0.01$.

reasons why HCWSE contained in cubosomal suspension had a high inhibitory effect on the development of AD-like skin lesion.

Conclusion

MO cubosomal suspension and EPC liposomal suspension, each containing HCWSE, were prepared by a sonication and a film hydration method, respectively. Compared with HCWSE solution in PBS, the lipid carrier suspensions, especially the cubosomal suspension, were efficacious preparations in terms of enhancing the *in vitro* skin permeation of HCWSE. The order of lymphocyte (B-cell, Th1 cell and Th2 cell) – modulating effect in mice having DNCB-induced AD-like skin lesion was HCWSE contained in cubosomal suspension > HCWSE contained in liposomal suspension > HCWSE dissolved in PBS. It is believed that the cubosomal suspension containing HCWSE would be an efficacious preparation for the treatment of AD.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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