Original Article

Complementary effects of white rose petal extract on canine atopic dermatitis

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Abstract

This study aimed to investigate the therapeutic efficacy of white rose petal extract (WRPE) in canine atopic dermatitis (CAD). Experiment 1 involved clinical assessments using atopic dogs and experiment 2 used beagles with experimentally induced dermatitis. The atopic dogs were divided into four groups: 1% WRPE solution (group 1), 0.5% WRPE solution (group 2), 1% WRPE cream (group 3) and 1% WRPE cream containing lipid granules (group 4). Three normal beagles were used for the experimental induction of dermatitis. Experimental dermatitis was induced by intradermal injections of histamine dihydrochloride, compound 48/80 or substance P. WRPE solution or cream was applied once daily in the experiment 1. 1% WRPE solution (group 5), 1% WRPE cream (group 6) and control cream (0%, group 7) were applied thrice daily for 28 days in experiment 2. In experiment 1, clinical assessments were performed by measuring transepidermal water loss (TEWL), lesion severity and pruritus. In experiment 2, the TEWL and wheal diameter were measured. Furthermore, histopathology and transmission electron microscopy were performed. In experiment 1, 1% WRPE decreased the TEWL and lesion severity ($P < 0.05$), whereas pruritus remained unchanged. In experiment 2, the increase in TEWL and changes in lipid lamellae and corneocyte derangement were significantly lower and wheal diameter, edema and inflammatory cells also significantly decreased in 1% WRPE cream-treated lesions ($P < 0.05$). Therefore, WRPE may be beneficial on CAD by restoring skin barrier function and reducing inflammation.

Keywords: Canine atopic dermatitis, experimental dermatitis, skin barrier function, white rose petal extract

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Introduction

Canine atopic dermatitis (CAD) is a chronic hypersensitive inflammatory skin disease that affects approximately 10% of dogs worldwide (Hillier and Griffin, 2001). Currently, medications with good evidence of high efficacy for CAD include topical and systemic glucocorticoids, calcineurin inhibitors like oral ciclosporin and Janus kinase 1 inhibitors like oclacitinib (Olivry *et al*., 2015). However, long-term management of chronic patients and the use of high-dose drugs in severe cases may be associated with significant adverse effects, including cutaneous atrophy, calcinosis cutis, polyuria/polydipsia, hepatotoxicity and other systemic signs of steroid potency, such as vomiting and diarrhea. Additionally, opportunistic infections, often associated with the use of ciclosporin, can lead to otitis, vomiting, diarrhea and urinary tract infections, often resulting from oclacitinib administration (Olivry *et al*., 2010; Olivry *et al*., 2015). These adverse effects should be considered when a long-term therapeutic drug use is indicated. Furthermore, the search for CAD therapeutics that are not associated with known negative side effects or long-term tolerance is vital.

The rose (genus *Rosa*) is an ornamental plant often grown in farms and gardens. Many *Rosa* species have long been used in herbal and folk medicines for various indications, as well as in cosmetic and perfume ingredients. For example, *Rosa* (*R.*) *rugosa* and *R. davurica* have been used in traditional medicine to treat diabetes mellitus, pain and chronic inflammatory diseases (Cho *et al*., 2003; Ng *et al*., 2005). Furthermore, our recent studies have demonstrated that *R. hybrida* petal extracts containing high amounts of polyphenols, flavonoids, tannins and proanthocyanins have strong antioxidant and anti-inflammatory properties (Park *et al*., 2009; Lee *et al*., 2011; Yon *et al*., 2018; Choi *et al*., 2019; Jo *et al*., 2021). Notably, extracts from white rose petals have anti-allergic effects *in vitro* and *in vivo*, such as in systemic and local hypersensitivity models, including those of atopic dermatitis (Jeon *et al*., 2008; Kwon *et al*., 2008; Jeon *et al*., 2009). Specifically, white rose petal extract (WRPE) inhibited degranulation from mast cells and markedly reduced the serum histamine, IgE, and interleukin-4 concentrations. So, WRPE effectively decreased systemic anaphylactic reactions and inhibited epidermal and dermal lesions including infiltration of inflammatory cells.

However, there have been no studies on the anti-inflammatory and anti-allergic (anti-atopic) properties of WRPE in dogs. Therefore, we investigated whether WRPE had anti-inflammatory or anti-allergic effects on CAD.

Materials and Methods

Study design: This study consisted of two experiments. Experiment 1 was a clinical assessment using atopic dogs to identify the alleviative effects against clinical signs of atopic dermatitis, and experiment 2 used dogs with experimentally induced dermatitis to investigate the protective effects against lesions caused by inflammatory reagents. Just before experiment 2, a pilot study was conducted to determine the appropriate doses of inflammatory reagents to induce experimental dermatitis. All experiments were approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Research Center (Approval #: CBNUA-1134-17-02, date: September 1, 2017).

Animals: In experiment 1, a total of 17 dogs diagnosed with CAD based on clinical history, typical and ongoing clinical signs, fulfillment of at least five Favrot diagnostic criteria (Favrot *et al*., 2010) and positive results on intradermal skin tests were enrolled. They had focal cutaneous lesions, such as erythema, lichenification and excoriation/alopecia. Other possible pruritic diseases were ruled out through adequate tests and treatments. A food elimination trial was performed using a commercial hydrolyzed hypoallergenic diet (Hypoallergenic; Royal Canin, Aimargues, France) for at least 8 weeks to rule out adverse cutaneous food reactions. Atopic dogs' medications were continued for at least one week for antimicrobial drugs and at least 4 weeks for glucocorticoids, oclacitinib or ciclosporin before study enrollment. Drug regimens remained unchanged throughout the study. All owners signed a consent form prior to the study's initiation.

In experiment 2, three normal, sexually intact, female beagles (median age, 2.5 years) were used. These dogs were chosen based on a lack of clinical or historical evidence of allergic skin disease, cutaneous bacterial infections or systemic disease. Each dog was housed separately in a different cage and fed commercially available food pellets (Science Diet; Hill's Pet Nutrition, Topeka, KS, USA).

Plant materials and extracts: The WRPE used in this study was conducted as previously described (Choi *et al*., 2015). *R. hybrida* flowers were purchased from a local farm, dried completely under sunlight and ground in a cutter mill. Powdered petals (10 g) were mixed with 250 mL of 70% ethanol and hot water in 1 L glass bottles. The bottles were placed in a shaking water bath set at a speed of 120 rpm and the desired temperature (25–75°C). After extraction, the petals were filtered using Whatman filter paper no. 4 (Whatman Inc., USA) with a vacuum pump (BOC International Ltd., London, UK). The extracts were vacuum evaporated on a rotary evaporator (BÜCHI, Switzerland) in a water bath at 40°C until the ethanol was removed and then the extracts were freeze-dried at −60°C for 24 h using a freeze-dryer (Ilshinbiobase Co., Ltd., Korea). This WRPE was prepared in 0.5% and 1% solutions and 1% cream for application to cutaneous lesions.

Topical WRPE-containing agents: The diluent (pH 7.0) for 1% and 0.5% WRPE solution contained glycerin, cetearyl alcohol, cetearyl glucoside and cellulose gum. Afterwards, 0.5% and 1% WRPE were added to each diluent.

The base cream (pH 7.0) contained glycerin, sodium hyaluronate, tocopheryl acetate, glyceryl acrylate/acrylic acid copolymer, propylene glycol, caprylhydroxamic acid, caprylyl glycol and disodium EDTA. A 1% WRPE cream was prepared by including 1% WRPE in this base cream.

The lamellar body (pH 7.0) contained cetyl alcohol, cholesterol, hydrogenated lecithin, phytosphingosine, stearic acid, oleic acid, lactic acid, caprylyl glycol, caprylhydroxamic acid, glycerin, polysorbate 60, steareth-2, steareth-21, polyethylene glycol-30 dipolyhydroxystearate, isohexadecane and caprylic/capric triglyceride. A 1% WRPE cream with a lamellar body was made by adding 1% WRPE, sodium hyaluronate, tocopheryl acetate, glyceryl acrylate/acrylic acid copolymer, propylene glycol, caprylhydroxamic acid, caprylyl glycol and disodium EDTA.

Experiment 1: This experiment was performed to identify the alleviative effects of WRPE in clinical applications. The two most severe cutaneous lesion sites were selected on each atopic dog for topical treatment and clinical assessment. Four types of topical agents (group 1, 1% WRPE solution, n = 4; group 2, 0.5% WRPE solution, n = 4; group 3, 1% WRPE cream, n = 4; group 4, 1% WRPE cream with lipid granules, n = 5) were epicutaneously applied to two selected lesions once daily for four weeks; the solution and cream were applied at 1 mL/cm^2 and 1/16th of an inch (1–2 mm).

The treated area was assessed for transepidermal water loss (TEWL), pruritus using a pruritus visual analogue scale (PVAS) and lesion severity at days 0, 14, and 28 after topical application.

Prior to measuring TEWL, the atopic dogs were allowed to acclimatize to test room conditions (20–21°C of temperature and relative humidity of 44%–66%). TEWL was measured using an unventilated closed-chamber device (VapoMeter; Delfin Technologies Ltd., Kuopio, Finland) according to the manufacturer's instructions. To reduce variability, five sequential measurements were taken at each measurement site and the middle three results were averaged (Plessis *et al*., 2013). All results are expressed as percentages between the detected results and the baseline value.

Pruritus severity was measured using PVAS scores (Cosgrove *et al*., 2013). The PVAS consisted of a 10-cm line with verbal descriptors evenly spaced at 2 cm intervals, with "normal dog" and "extremely severe itching" defined at 0 and 10 cm, respectively. Owners were asked to assess the severity of their dog's itch along this scale.

The extent and severity of cutaneous lesions were scored according to the modified canine atopic dermatitis extent and severity index (CADESI)-4 (Olivry *et al*., 2014). Briefly, each lesion score was generated by calculating the sum of three items (erythema, lichenification, and excoriations/alopecia) on a scale of 0 (none), 1 (mild), 2 (moderate) and 3 (severe).

Experiment 2: This experiment was performed to identify the protective effects of WRPE against lesions induced by inflammatory reagents. First, a pilot experiment was conducted to identify the optimal substance concentration for the generation of atopic lesions (data not shown). According to preliminary results, a volume of 0.1 mL of 5% histamine dihydrochloride (CAS, 56-92-8), 10 mg/mL of compound 48/80, and 100 µM of substance P acetate hydrate most optimally created reagent-induced dermatitis via intradermal injection. All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The groups of experiment 2 were classified as follows: group 5 (1% WRPE solution), group 6 (1% WRPE cream), and group 7 (control cream [0%]). Initially, three experimental beagle dogs were shaved bilaterally on their thorax. To indicate the injection sites for inflammatory reagents, a grid was marked on each side of the experimental dogs using an indelible marker. There were two histamine dihydrochloride (5%) injection sites on the left thorax and one each for compound 48/80 (10 mg/mL), substance P (100 µM), histamine phosphate (0.1 mg/mL; the positive control; national drug code, 0268-0248-05; ALK-Abello Pharm., Inc., Mississauga, Ontario, Canada) and sterile water (the negative control): of the two histamine dihydrochloride (5%) injection sites, one was for biopsy on day 2 and the other one was for non-invasive measurement (day 0, 2, and 8) and biopsy on day 8 and the all agents were intradermally injected at a dose of 0.1 mL per site. Additional two compound 48/80 injection sites were also created on the right thorax to perform transmission electron microscopy (TEM). Three types of topical agents (1% WRPE solution or 0% or 1% WRPE cream with lipid granules) were epicutaneously applied to the reagent-induced lesions thrice a day for eight consecutive days. As in experiment 1, the solution was applied at a dose of 1 mL/cm^2 and the cream was applied at a thickness of approximately 1/16th of an inch (1–2 mm). In total, three inflammatory sites were created by intradermal injection of each reagent on days 1, 4 and 7 after the initial application of the topical agents.

TEWL and wheal diameter measurements were taken four times for reagent-induced lesions before topical treatment and 15 mins after injection with inflammatory reagents.

In each of the three experimental dogs, skin specimens were collected on days 0, 2 and 8 from histamine and compound 48/80-induced lesion sites via punch biopsy (8-mm biopsy punch; Integra Miltex Inc., York, PA, USA). Histamine-induced lesions were used for the histopathological analysis. Briefly, biopsy specimens were fixed in neutral buffered 10% formalin, bisected, embedded in paraffin and sectioned and stained with hematoxylin and eosin (H&E), as done routinely. Inflammatory cells, including neutrophils, eosinophils, lymphocytes, plasmocytes, macrophages, and mast cells were counted in the dermis of each section using a light microscope (Eclipse Ci; Nikon, Tokyo, Japan). Five consecutive dermal fields were examined and the average inflammatory cell count was calculated. All cell counts are expressed as the number of cells in the high-power field.

The compound 48/80-induced lesion specimens were fixed overnight in phosphate-buffered saline containing 2.5% glutaraldehyde at 4°C (pH 7.4). Tissues were washed in 0.1 M cacodylate buffer (pH 7.4) and post-fixed in 1% osmium tetroxide for 2 h. The specimens were then dehydrated with a graded ethanol solution, impregnated with propylene oxide, and embedded in EPON resin. Tissue blocks from each lesion were sectioned at 70 nm, perpendicular to the

skin surface. The sections were collected on copper grids and stained with 7% uranyl acetate in methanol and lead citrate. The samples were then examined using TEM (JEM-2100; JEOL, Tokyo, Japan).

Five electron micrographs were taken from the lower and middle *stratum corneum* at 6,000×–30,000× magnification. The *stratum corneum* lipid lamellae were graded on a semiquantitative scale (Inman *et al*., 2001; Jung *et al*., 2013). The thickness (number of bilayers) of lipid deposition at sites where lipids were present was estimated as follows: 1, few lipid bilayers; 2, intermediate between 1 and 3; 3, moderate number; 4, intermediate between 3 and 5; and 5, high number. The continuity of lipid deposition was scored on each micrograph as follows: 1, very patchy and interrupted lipid layers; 2, intermediate between 1 and 3; 3, some interruption in the lipid layers; 4, intermediate between 3 and 5; and 5, continuous uninterrupted lipid layers. Mean scores for thickness and continuity across the five photomicrographs were also calculated, with the values of these two measurements added together. Corneocyte arrangement was assessed according to the following scale: 1, very disorganized arrangement and widened intercellular space; 2, intermediate between 1 and 3; 3, mild disorganization and widened intercellular space; 4, intermediate between 3 and 5; 5, organized arrangement and constant intercellular space.

Considering the invasive characteristics of biopsy and the experimental cost, H&E staining was performed only on the histamine-induced group that showed significant improvement in the wheal lesion. Additionally, for the same reasons as H&E staining, TEM was also performed only on the compound 48/80-induced group that showed significant improvement in TEWL.

Statistical analyses: Data was analyzed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). A Friedman test was used to evaluate the data within each group for changes over time. Differences between groups were tested using the Kruskal-Wallis test. Dunn's test was used for post-hoc pairwise comparisons. All values in each graph are expressed in means ± standard deviations. Differences were considered significant at $p < 0.05$.

Results

Experiment 1: During the study period, 17 dogs from five breeds were assessed clinically after the application of WRPE. The dogs were divided into four groups based on the agents applied: 1% WRPE solution (group 1, n = 4), 0.5% WRPE solution (group 2, n = 4), 1% WRPE cream (group 3, n = 4), and 1% WRPE cream containing lipid granules (group 4, n = 5). The site and type of the two selected cutaneous lesion sites for each dog are summarized in Table 1.

Table 1 Characteristics of atopic dogs and their cutaneous lesions.

Group	Breed	Gender	Age (years)	Lesion site (type)
1	Maltese	Male	8	Left pinna (erythema) Right pinna (erythema)
	Cocker Spaniel	Male	6	Left pinna (erythema) Right pinna (erythema)
	Dachshund	Female	4	Left cubital flexor (lichenification, alopecia) Right cubital flexor (lichenification, alopecia)
	Maltese	Female	8	Left pinna (erythema, lichenification) Right pinna (erythema, lichenification)
2	Shih Tzu	Male	12	Left cubital flexor (lichenification) Right cubital flexor (lichenification)
	Shih Tzu	Female	12	Left inguinal area (erythema) Right inguinal area (erythema)
	Boston Terrier	Male	2	Left cubital flexor (erythema, excoriation) Right cubital flexor (erythema, excoriation)
	Shih Tzu	Female	11	Left inguinal area (erythema) Right inguinal area (erythema, excoriation)
3	Cocker Spaniel	Male	3	Left pinna (erythema, lichenification) Right pinna (erythema, lichenification)
	Shih Tzu	Female	15	Left pinna (erythema, lichenification) Right pinna (erythema, lichenification)
	Cocker Spaniel	Male	6	Left inguinal area (erythema, lichenification) Right inguinal area (erythema, lichenification)
	Maltese	Male	8	Left pinna (erythema, lichenification) Right pinna (erythema, lichenification)
4	Boston Terrier	Male	2	Left inguinal area (erythema) Right inguinal area (erythema)
	Shih Tzu	Female	11	Left inguinal area (erythema) Right inguinal area (erythema)
	Shih Tzu	Female	12	Left pinna (erythema, lichenification) Right pinna (erythema, lichenification)
	Dachshund	Female	4	Left cubital flexor (lichenification, alopecia) Right cubital flexor (lichenification, alopecia)
	Maltese	Female	3	Left cubital flexor (erythema) Right cubital flexor (erythema)

Among the four groups, the TEWL values did not differ at each measurement ($P > 0.05$, Figure 1A). After topical application of WRPE, the TEWL in group 1 significantly decreased ($P < 0.001$) and TEWL on day 28 was significantly lower than that on day 0 ($P = 0.01$). While TEWL showed a tendency to decline in group 4, this difference was not significant ($P = 0.09$).

Among the four groups, the levels of pruritus did not differ at each measurement days ($P > 0.05$, Figure 1B). After topical application of WRPE, PVAS did not significantly change across time points ($P > 0.05$).

Among the four groups, modified CADESI-4 scores did not differ at each measurement days ($P > 0.05$); however, the lesion severity in group 4 significantly decreased with time ($P = 0.02$, Figure 1C). Although the difference between days 0 and 14 was not significant in group 4 ($P = 0.81$), the score on day 28 tended to be lower than that on day 0 ($P = 0.08$).

Experiment 2: Experiment 2 used beagles with experimentally induced dermatitis and WRPE was topically administered to reagent-induced lesion sites as follows: 1% WRPE solution (group 5), 1% WRPE cream (group 6) or control cream (group 7; 0% WRPE) (Figure 2).

TEWL values for all reagent-induced lesions increased on days 1, 4 and 7 following the induction of experimental dermatitis (Figure 3A). Between lesions resulting from histamine dihydrochloride, compound 48/80 and substance P, these increases were most prominent in compound 48/80-induced lesions ($P < 0.01$). Although values at day 7 were significantly higher than those at day 0 ($P < 0.01$), the values in group 6 were lower than those in group 7 for compound 48/80-induced lesions ($P = 0.04$). There were no other significant differences among the three groups after histamine dihydrochloride or substance P-induced lesions on any experimental day ($P > 0.05$).

While wheal diameter did not differ among the three types of induced lesions on any experimental day (Figure 3B), 1% WRPE cream showed decreased histamine dihydrochloride-induced lesion sizes on day 7 compared to those on day 1 ($P = 0.01$).

Numerous inflammatory cells, desquamation of the *stratum corneum* and dermal edema were noted in the histamine dihydrochloride-induced lesions (Figure 4A). In all groups, cell infiltration intensified over time ($P = 0.03$; Figure 4). Cell numbers in the three groups significantly increased on day 8 when compared with those on day 0 ($P < 0.05$, Figure 4B). Inflammatory cells in group 6 were significantly lower than those in group 7 on days 2 and 8 ($P < 0.001$).

Disruption of lipid bilayers, corneocyte disarrangement and increased intercorneocyte space were observed in compound 48/80-induced lesion tissues (Figure 5A). In the three groups, the diminution of thickness and continuity of lipid deposition and the disruption of corneocyte arrangement appeared aggravated over time ($P = 0.03$, Figure 5). TEM scores for all groups were significantly decreased on day 8 when compared with those on day 0 ($P < 0.05$, Figure 5B). Group 6 showed significantly fewer disruptions of lipid lamellae and corneocyte arrangement than group 7 ($P < 0.05$).

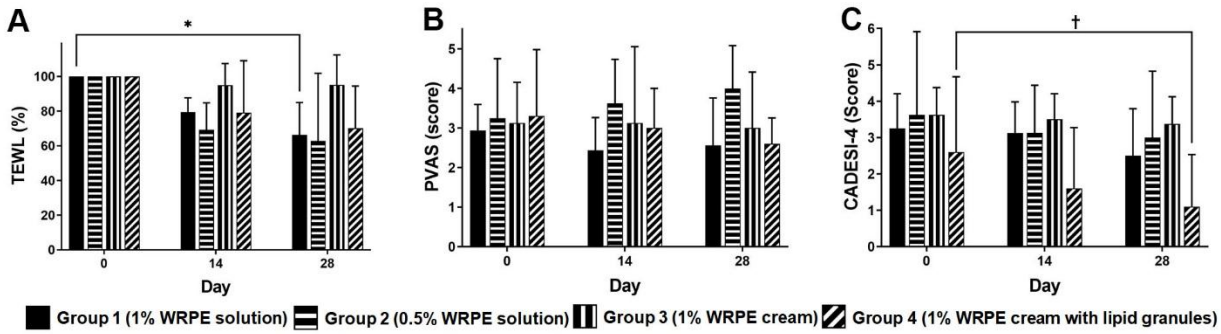

Figure 1 Changes in TEWL percentage (A), pruritus (B) and modified CADESI-4 scores (C) following topical application of WRPE in experiment 1 using atopic dogs. (A) The TEWL in group 1 significantly decreased ($P < 0.001$), with TEWL on day 28 significantly lower than that on day 0 (*$P = 0.01$). (B) The level of pruritus was not influenced or changed by the factor of time or topical treatment applicants ($P > 0.05$). (C) The lesion severity in group 4 significantly decreased with time ($P = 0.02$) and the score on day 28 tended to be lower than that on day 0 (†$P = 0.08$). Values are expressed as means ± standard deviations. Group 1: 1% WRPE solution, Group 2: 0.5% WRPE solution, Group 3: 1% WRPE cream, Group 4: 1% WRPE cream containing lipid granules. CADESI-4, canine atopic dermatitis extent and severity index-4; TEWL, transepidermal water loss; WRPE, white rose petal extracts.

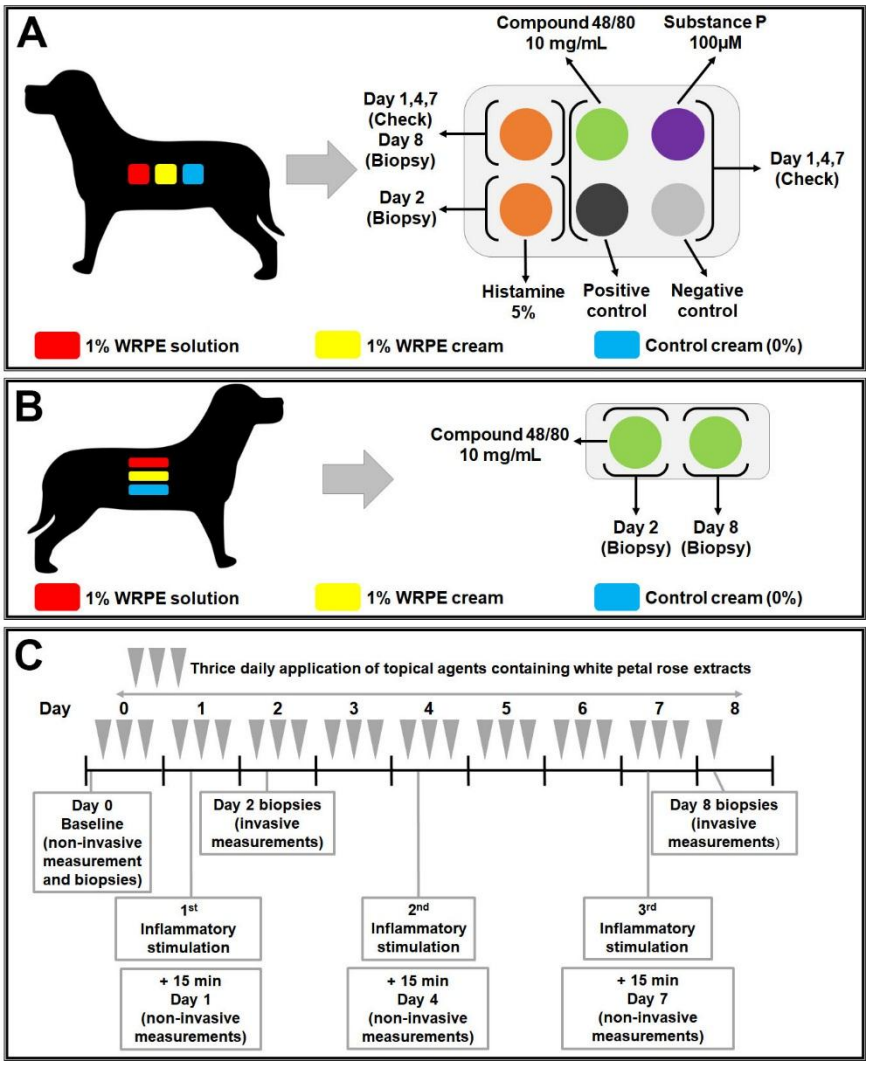

Figure 2 Schematic representation of experimental sites (A and B) and experimental timeline for reagent-induced dermatitis (C) in experiment 2. (A and B) Clipping of the hair coat was performed on the bilateral thorax. Three reagents were injected intradermally for induction of experimental dermatitis and three types of topical agents (1% WRPE solution, 1% WRPE cream, or 0% cream) were epicutaneously applied to the reagent-induced lesions. TEWL measurements and wheal diameter, as well as biopsies for histopathology were performed on the left-side of the thorax (A). For TEM assessments, biopsy specimens were collected from the right side of the thorax (B). (C) Inflammatory stimuli were introduced via intradermal injection of histamine dihydrochloride, compound 48/80 or substance P at days 1, 4 and 7. A topical agent was epicutaneously applied to the experimental lesion sites three times daily for 8 consecutive days. Non-invasive measurements (TEWL and wheal diameter) were performed four times before topical treatment and 15 mins after each inflammatory reagent injection. Skin specimens for invasive assessments (histopathology and TEM) were collected three times before and at days 2 and 8 after topical treatment administration. TEM, transmission electron microscopy; TEWL, transepidermal water loss; WRPE, white rose petal extracts.

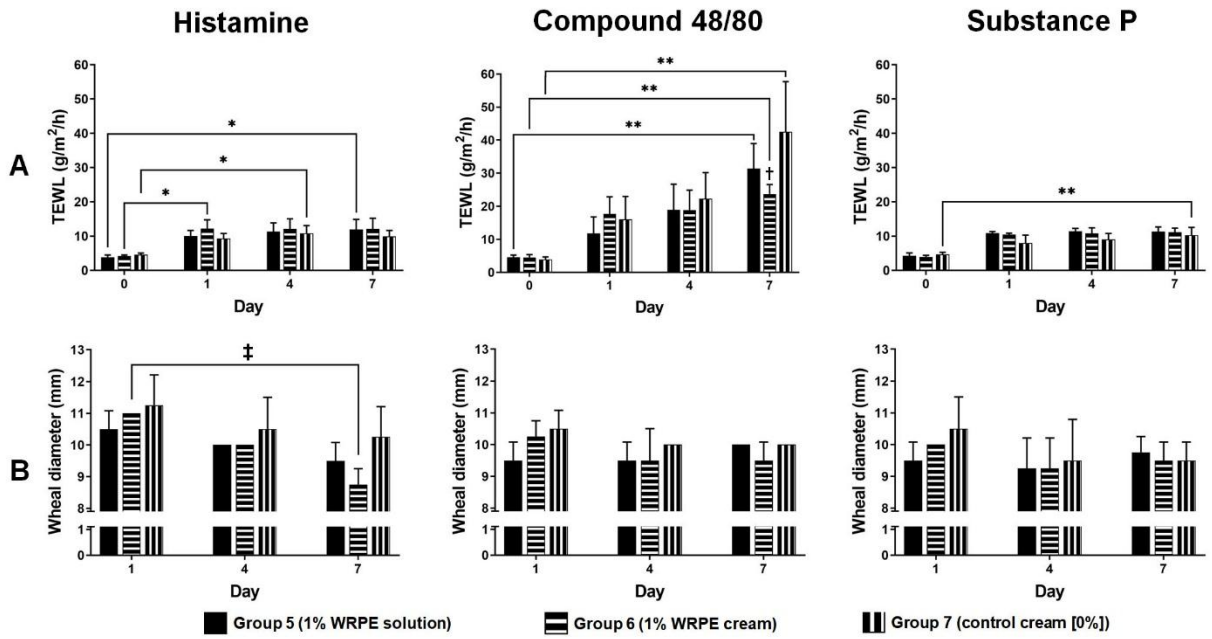

Figure 3 Changes in TEWL (A) and wheal diameter (B) following topical application of WRPE in experiment 2 using beagle dogs with histamine dihydrochloride, compound 48/80 or substance P-induced dermatitis. (A) TEWL values for reagent-induced lesions generally increased by days 1, 4 and 7. Especially these increases were most prominent in compound 48/80-induced lesions ($P < 0.01$). Although values at day 7 were significantly higher than those at day 0 (**$P < 0.01$), values in group 6 were lower than those in group 7 for compound 48/80-induced lesions (†$P = 0.04$). (B) 1% WRPE cream significantly decreased the size of histamine dihydrochloride-induced lesions on day 7 when compared with that on day 1 (‡$P = 0.01$). Values are expressed as means ± standard deviations. *$P < 0.05$ compared with day 0. **$P < 0.01$ compared with day 0. †$P = 0.04$ compared with TEWL of compound 48/80 on day 7. ‡$P = 0.01$ compared with day 0. Group 5, 1% WRPE solution; Group 6, 1% WRPE cream; Group 7, control cream (0%). TEWL, transepidermal water loss; WRPE, white rose petal extracts.

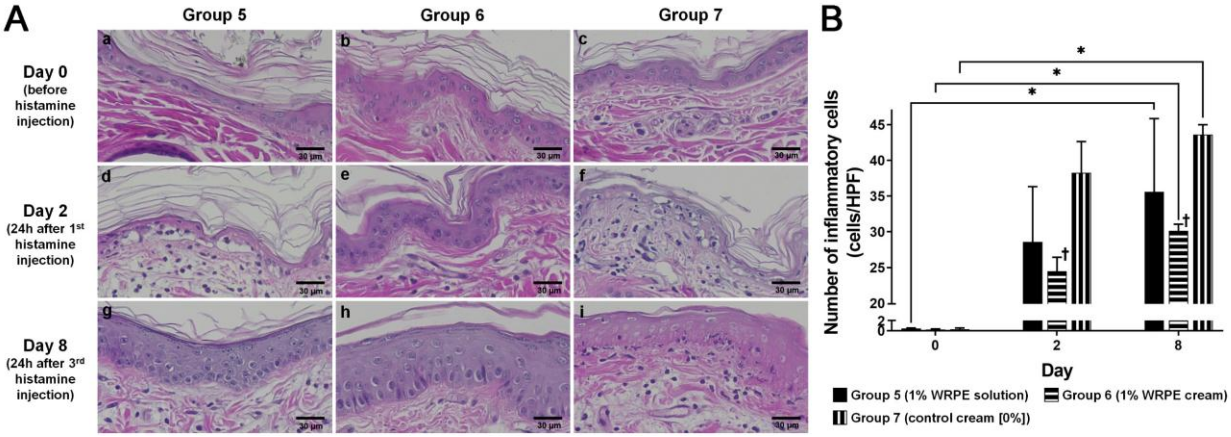

Figure 4 Changes in microscopic features (A) and inflammatory cell number (B) of histamine dihydrochloride-induced dermatitis following topical application of WRPE in experiment 2 using a beagle dog. (A) In all groups, numerous inflammatory cells, desquamation of *stratum corneum* and dermal edema were noted on day 2 (b, e, and h) and day 8 (c, f, and i). These inflammatory changes were the least in group 6 (e and f). All were hematoxylin-and-eosin-stained. (B) In three groups, the cell number increased over time ($P = 0.03$). Cell numbers in the three groups significantly increased on day 8 when compared with those on day 0 (*$P < 0.05$). The inflammatory cell count in group 6 was significantly lower than that in group 7 on days 2 and 8 (†$P < 0.001$). Values are expressed as means ± standard deviations. Group 5, 1% WRPE solution; Group 6, 1% WRPE cream; Group 7, control cream (0%). WRPE, white rose petal extracts.

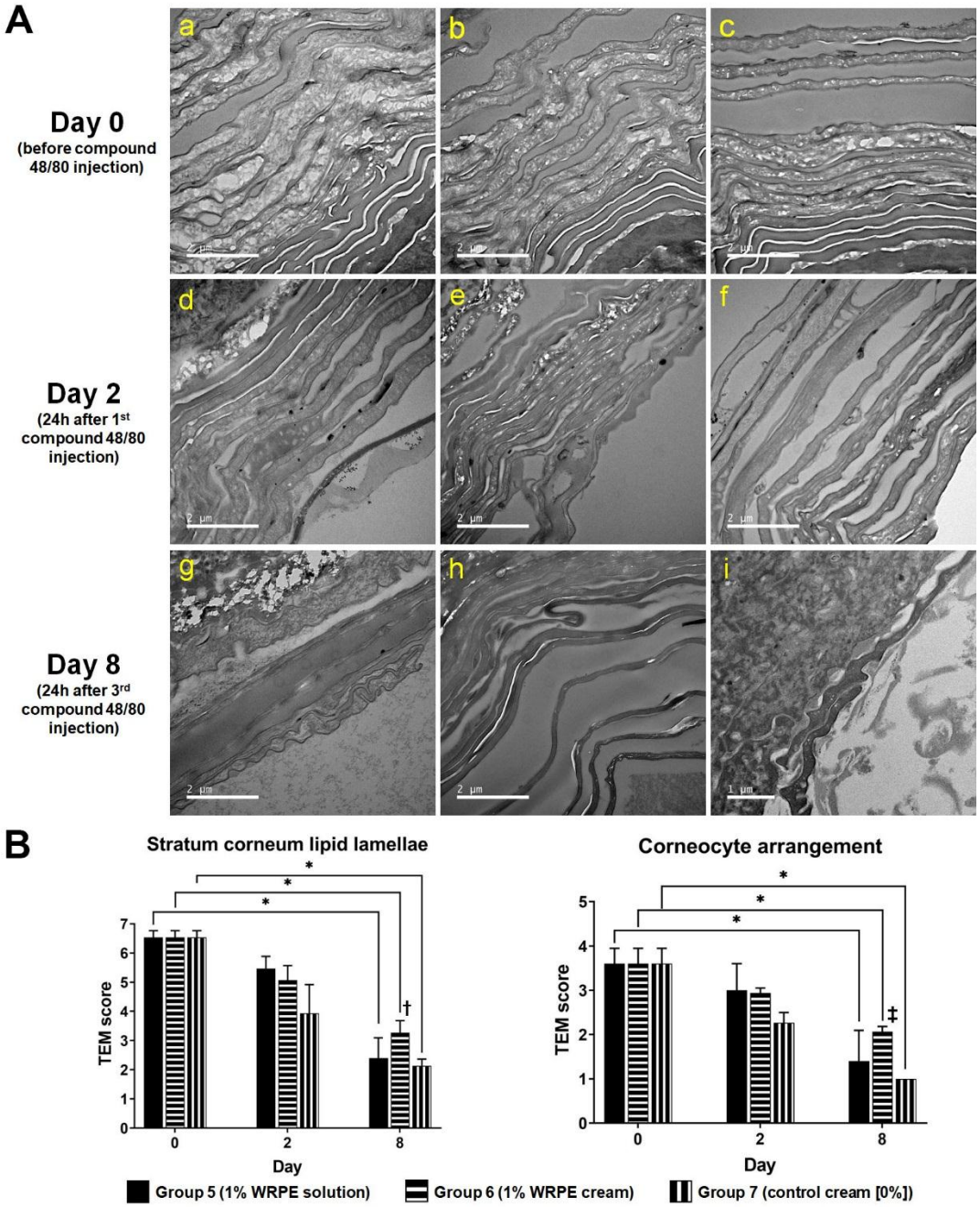

Figure 5 Changes in *stratum corneum* lipid lamellae and corneocyte arrangement of compound 48/80-induced dermatitis following topical application of WRPE in experiment 2 using a beagle dog. (A) On TEMs of all groups, lipid bilayer destruction, corneocyte disarrangement and increased intercorneocyte space were observed at day 2 (b, e, and h) and day 8 (c, f, and i). These changes were the least in group 6 (e and f). Osmium tetroxide was given after fixation. (B) TEM scores decreased over time in each of the three groups ($P = 0.03$). Group 6 showed significantly fewer disruptions of lipid lamellae (†$P = 0.03$) and corneocyte arrangement (‡$P = 0.02$) than did group 7. Values are expressed as means ± standard deviations. *$P < 0.05$ compared with day 0. Group 5, 1% WRPE solution; Group 6, 1% WRPE cream; Group 7, control cream (0%). TEM, transmission electron microscopy; WRPE, white rose petal extracts.

Discussion

The present study revealed the complementary effects of WRPE in restoring skin barrier function and reducing inflammation in clinical cases and in a canine model of atopic dermatitis. WRPE lessened the epidermal water loss in atopic and experimental dermatitis. Additionally, it reduced inflammatory cell infiltration, desquamation and dermal edema.

The activation of mast cells and T helper cells plays an important role in the pathogenesis of CAD (Loewenstein and Mueller, 2009). Degranulation of mast cells results in the release of histamine, which is related to the immediate phase of allergic inflammation, including vasodilation and dermal edema (Becker et al., 1988). Because the canine skin functions as a biophysical barrier between the environment and the body (Cornegliani *et al.*, 2012), impairment of this barrier can have a critical impact on the etiology of skin diseases, including CAD (Marsella, 2012). Skin barrier function is dependent on structural integrity, which is maintained by corneocytes and intercellular lipids in the *stratum corneum* (Nemes and Steinert *et al.*, 1999). In CAD, penetration of

environmental allergens is enhanced through the *stratum corneum* due to alterations in its structural integrity, thus increasing allergic sensitization and inflammatory processes, which further impair skin barrier function (Marsella *et al*., 2010).

WRPE may downregulate the inflammatory process in atopic lesions through various mechanisms. WRPE reduced the serum IgE, histamine and interleukin-4 levels in a mouse model (Jeon *et al*., 2008) and pretreatment effectively inhibited histamine-induced vascular permeability increase (Jeon *et al*., 2009). Additionally, WRPE affects the expression of type 1 and 2 cytokines in T helper cells (Kwon *et al*., 2008; Jeon *et al*., 2009). In the present study, 1% WRPE reduced TEWL and the size of the atopic lesions. Microscopically, inflammatory cell infiltration, desquamation of the *stratum corneum* and dermal edema were also diminished in the WRPE-treated lesions. TEM revealed minimal changes in the lipid lamellae and corneocyte arrangements in the WRPE-treated lesions. Therefore, although we did not identify the direct relevance between such mechanisms and our findings, WRPE may be beneficial on CAD by restoring skin barrier function and reducing inflammation.

It is believed that the broad antimicrobial activities of WRPE, especially on atopy-inducing *Staphylococcus*, might contribute partly to its anti-allergic effects (Park *et al*., 2016). Furthermore, plant pyrogallol compounds have recently been reported to be an anti-allergic source (Nakano *et al*., 2020). We confirmed a high amount of pyrogallol (1,2,3-benzenetriol) derivatives (43.6% of major peak areas in GC-MS analysis) in WRPE (Yang *et al*., 2013; Yon *et al*., 2018) which might be the main active ingredients for the alleviation of atopic reactions.

The unit of TEWL in the experiment 1 (percentage) and experiment 2 ($g/m^2/h$) was different. The most ideal comparison is to compare the absolute values among groups. However, there were some variations of measurement conditions (season, weather and species) despite the effort to reduce the variation in experiment 1, whereas there was no variation in experiment 2 (same environmental condition and species). So, the percentage was alternatively used to correct the effects caused by the variation of measurement conditions in experiment 1.

The present study has some limitations with respect to the further generalization of its findings. First, the effect of WRPE was not systemically evaluated in clinical patients via comparison with negative and placebo controls and other common CAD treatments. Second, histopathology was performed only in beagle dogs with experimental dermatitis because of the difficulty and invasiveness involved in acquiring biopsy samples from atopic dogs. Therefore, we were unable to study the influence of WRPE on naturalistic atopic lesions at the microscopic level. Third, the model of reagent-induced dermatitis used here differs from spontaneous atopic lesions in critical ways. Although experimental reagents, including histamine dihydrochloride, compound 48/80 and substance P, are commonly used to induce atopic-like lesions and pruritus in rodents, they do not reflect the complex pathogenesis and mechanisms of CAD. Fourth, because inflammatory agents such as histamine dihydrochloride, compound 48/80, substance P and positive control histamine were injected in the adjacent area, it was difficult to evaluate the specific-pruritus of each inflammatory agent-induced lesion. Lastly, the negative control for comparison in experiment 2 was not included. Comparison with a negative control is necessary to make a more accurate comparison; however, it was not performed. Therefore, more studies are needed in large populations of atopic patients, as is the use of an objectively verified model of CAD, such as *Dermatophagoides farinae* sensitization, to gain further validity (Marsella *et al*., 2006).

In conclusion, a product containing 1% WRPE may be used as an adjunctive treatment option as a safe and effective anti-atopic agent for CAD through reduction of inflammation and restoration of skin barrier function.

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