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Effects of Tomato Extracts on Detergent-Induced Dry Skin in Rats

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Dry skin, called xerosis as medical term, is one of the most common skin problems. Many epidemiological studies show that the consumption of foods containing lycopene plays an important role in protecting the epithelial tissue. In this study, water extracts of tomato (WET) containing lycopene were fed and applied to evaluate the effects on dry skin induced by kitchen detergent in rats. These effects were identified by protein analysis and histological changes such as inflammatory erythematic skin as well as acanthosis. The visual scoring for skin observation showed the value such as 4 indicating fiery red with edema after detergent application to skin for 3 wk. However, WET feeding and application to skin showed the decreased values, from 0.7 to 1.0. In addition, it was noteworthy that the epidermis of dry skin show apparent acanthosis with abnormally accentuated keratinization and parakeratosis. However, acanthosis was reversed by feeding and application of WET to dry skin. In order to analyze the effects of WET on dry skin induced by detergent, protein analysis was carried out. The increased amount of protein in dry skin after WET feeding and application would be suggested as one of biochemical mechanisms for recovering the damaged skin. Thus, it would be recommended that water-extracted tomato is a new ingredient in skin regeneration from dry skin induced by detergent.

Key words : Dry skin, inflammatory reaction, acanthosis, tomato extracts

Introduction

Dry skin, called xerosis as medical term, is one of the most common skin problems. Dry skin is a common skin condition characterized by roughness, scaling, loss of elasticity and often discomforting sensations of itching and burning. Dryness of the skin is a common condition in the elderly. As people age, the outer layer of skin loses water, causing the surface to become dry and rough [3]. In addition, dry skin is often a problem in cooler climates, especially during winter months when home heating systems are used regularly. This unhumidified heat is dry and draws moisture from the skin. It is defined as idiopathic or constitutional xerosis when it appears independently of age or any well-known pathological or physiopathological cause [14]. While the exact cause of xerosis is unknown, the condition is related to altered lipid and protein composition of the stratum corneum (SC), in addition to other changes in epidermal differentiation [1]. Especially, the hydration of the stratum corneum plays an important role in keeping the skin surface smooth and supple, whose reduction produces dry skin. However, it appears as though dry skin does not occur as a result of decreased water in the skin, but rather is the result of abnormal keritinization and desquamation [6].

In general, there is a twofold approach to the treatment of dry skin: (1) minimizing irritation and (2) moisturization [7,14]. Based on these approaches, creams and lotions as topical agents that contain keratolytic agents, such as urea, salicylate, lactic acid, vitamin A, and propylene glycols have been developed. In contrast to numerous studies on topical agents, there have been few studies reporting the effects of fruits, vegetables and food supplements on the SC functions [8,10,11]. Many epidemiological studies have showed that the consumption of foods containing lycopene plays an important role in protecting the epithelial tissue [12].

Especially, tomato (*Lycopersicon esculentum*) contains higher nutrients such as folate, vitamin A & C as well as carotenoids, particularly lycopene. This indicates the possible role of tomato in preventing and recovering dry skin caused by many different factors. In this study, tomato extracts were fed and applied to evaluate the effects on dry skin induced by kitchen detergent in rats. These effects were also identified by protein analysis and histological

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changes such as inflammatory erythematic skin as well as acanthosis.

Materials and Methods

Tomato extraction

Ripen cherry tomato (*L. esculentum* var. *cerasiforme*) produced from Bit-nae farm in Kim cheoun, Korea was used as extracts to apply cutaneously. To get the water extract of tomatoes (WET), raw tomatoes without a stalk were washed with water and ground with grinder. A 500 g tomato with 1,000 ml DW was in water bath at 60°C for 5 hr (SWB-20 shaking water bath, Jeio Tech) and filtered. The tomato liquid was centrifuged to remove any contaminants and then dried by freeze-drier.

Animal and group classification

All animals were used five weeks old rat of S.D. (Spraque Dawley). Animals had 12 hr of daylight and 12 hr darkness, and the environmental temperature was constantly maintained at 20±2°C. The rats were kept in cages one by one and were given usual solid diet and water. After adapting rats to new circumstances, experimental rat groups were classified into 5 groups (6 animals per group) as shown in Table 1.

Dry skin induction and WET application

The dorsal part of rats were shaved in size of 4×5 cm by shaving machine. And then the 0.2 ml of kitchen detergent with sterilized cotton pad was applied daily to create the dry skin for 3 wk. The 1.5 g of WET powder was mixed with 30 ml DW. For 2 wk after dry skin induction, the mixed liquid of WET as amount as 5% of body weight was fed and applied daily to rats with dry skin induced by the detergent.

Protein analysis

Skin samples were collected weekly for protein analysis. The skin of 2 g was homogenized in the 20 ml homogenizer buffer (0.25 M sucrose:0.1 EDTA=1:1) and centrifuged twice for 15 min at 5,000 rpm. Protein from skin homogenate was denatured in boiling water for 5 min and added to 12% SDS-PAGE. Protein analysis of skin sample was achieved by sodium dodecyl sulfate polyarylamide gel electrophoresis on the 5% Stacking gel and 12% separating gel. The gel was stained with coommassie brilliant blue R-250. Marker protein (myosin: 198,000, ß-galactosidase: 115,000, bovine serum albulmin: 90,500, glutamate dehydrogenase: 61,500, ovalbmin: 46,200, carbonic anhydrase: 37,800, myoglobin: 26,000, lysozyme: 18,500, aprotinin: 9,000 Da) was used.

Morphological changes

A skin around 5 mm was taken from the dorsal of rats, and was fixed with formalin and embedded by paraffin. Six-micrometer thick sections were cut from a few different areas of each sample by microtome, and they were stained with hematoxylin and eosin (H & E). Image acquisition and analysis of skin tissues were visualized under a Carl Zeiss Axioplan 2 microscope (Carl Zeiss Canada Inc. Toronto, ON, Canada). Images were captured with a Zeiss Axiocam digital camera connected to the microscope using AxioVision 3.0 software. Analysis of the entire sections to assess epidermis was performed on images collected in series and magnified 20X.

Results and Discussion

Erythema with rough and scaly skin is common symptom in various dermatosis and dermatitis characterized by the dry skin, such as senile xerosis, atopic dermatitis and seasonal xerosis in winter [5,13]. Irritant contact dermatitis is one of the main occupational disease among health care workers and usually shows rough and scaly skin accompanied with erythematic and burning sensation [5]. The damaged skin is mainly explained by hand washing and

Table 1. Group classification

Groups	Characteristics
Group Ⅰ	Normal control
Group Ⅱ	Dry skin induced in rats by kitchen detergent for 3 wk
Group Ⅲ	Group II followed by feeding and application of WET for 2 wk
Group Ⅳ	Group II followed by application of WET on dry skin for 2 wk
Group Ⅴ	Group II followed by feeding WET for 2 wk

appears to be more severe when hard water is used [15]. Thus, erythematic skin has been used as one of important markers for dry skin caused by dishwashing detergent.

In this study, external skin observations related to erythematic skin induced by detergent are shown in Fig. 1. The inflammatory, erythematic and scaly skin was shown in all groups after detergent treatment for three weeks. The group II showed the skin with slightly decreased inflammation two weeks later after stopping detergent treatment (Fig. 1. A2). This seems to be due to natural recovery by stopping detergent treatment. However, the inflammatory, erythematic and scaly skin was not shown in group III, IV and V. This indicates that WET would be effective on the dry-skin caused by detergent.

Especially, the group III showed faster recovery from the damaged skin than those from group IV and V. This indicates that WET simultaneous feeding and skin application of WET would be more effective than feeding or skin application alone.

The effects of WET on dry skin were also expressed as visual scoring based on Frosch & Uehara method in Table 2. The visual scoring was estimated according to the level of Inflammatory reaction which was one of reasons for erythema. All scores for group I, group II, group III, group IV and group V were almost the same value such as 4+ indicating fiery red with edema, after application of detergent to skin for 3 wk. The values were slightly decreased to 2.8±0.41 in one week later without WET treatment as shown in Group II. However, the group III, IV and V treated with WET showed the decreased values, 0.7±0.52, 1.0±0.00 and 0.7±0.52, respectively. Especially, the group III and V showed lower values than that of group IV.

In addition, histological evaluation of the skin was car-

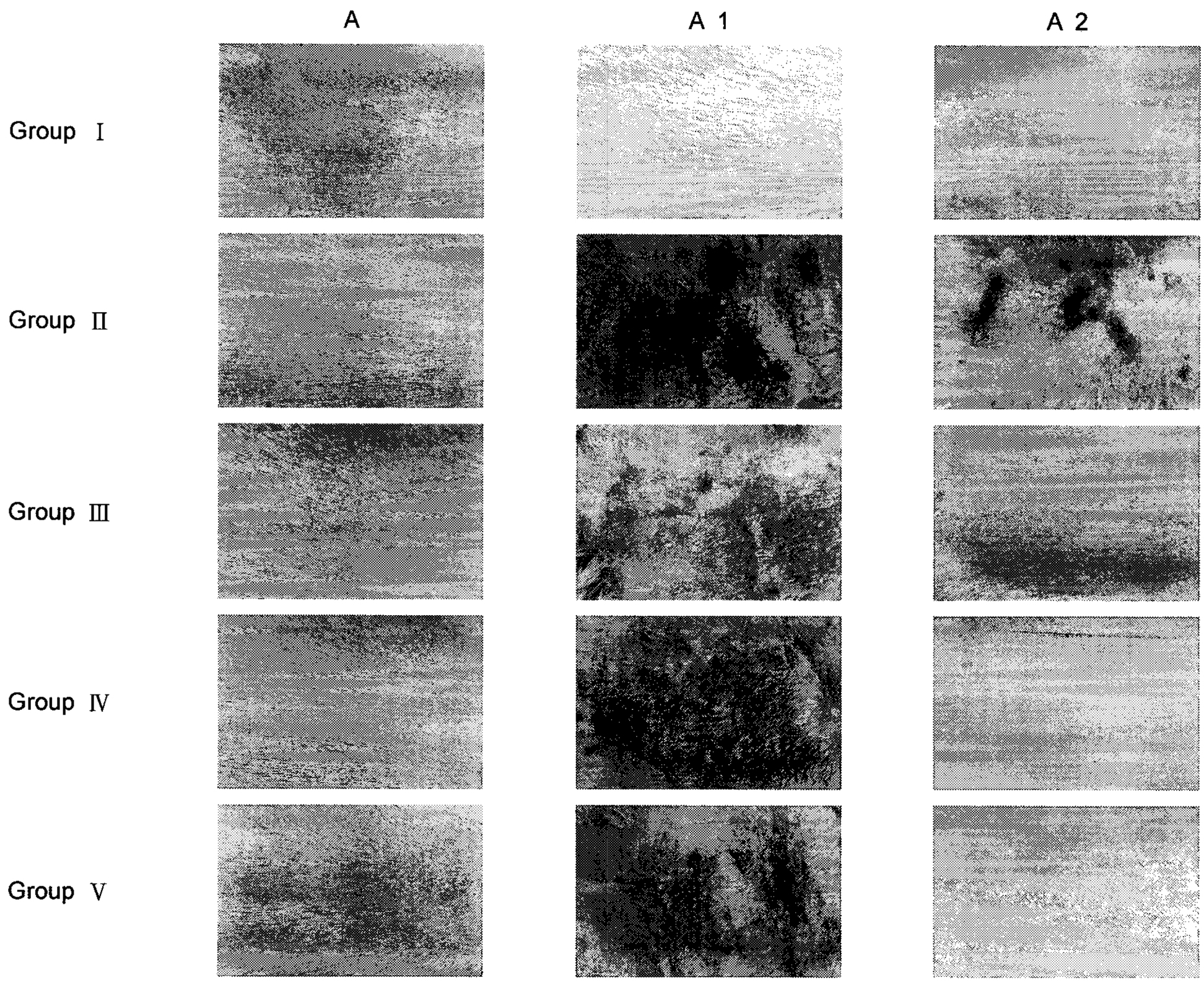

Fig. 1. Skin observations and WET effects on dry skin. A: Normal skin, A1: Dry skin induced by kitchen detergent for 3 wk, A2: Skin treated with WET for 1 wk except group II.

Table 2. The visual scoring and WET effects on dry skin induced by detergent (n=6)

Groups	Treatments	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean±SD
Group Ⅰ					No treatment			
Group Ⅱ	Detergent	4+	4+	4+	4+	4+	4+	4.0±0.00
	No WET	3+	3+	2+	3+	3+	3+	2.8±0.41
Group Ⅲ	Detergent	4+	4+	4+	4+	4+	4+	4.0±0.00
	WET-F&S	0+	0+	1+	1+	1+	1+	0.7±0.52
Group Ⅳ	Detergent	4+	4+	4+	4+	4+	4+	4.0±0.00
	WET-S	1+	1+	1+	1+	1+	1+	1.0±0.00
Group Ⅴ	Detergent	4+	4+	4+	4+	4+	4+	4.0±0.00
	WET-F	1+	1+	0+	0+	1+	1+	0.7±0.52

Skin observation measured by the visual scoring system expressed as 0+: no erythema, 1+: slight redness, spotty or diffuse, 2+: moderate redness, 3+: intense redness, 4+: fiery red with edema. WET-F&S; WET-feeding and skin application, WET-S: skin application only, WET-F: feeding only. The visual scoring was recorded at three weeks after detergent treatment and at one week after WET feeding and application.

ried out and is shown in Fig. 2. Group II, having dry skin induced by detergent for 3 wk showed acanthosis (increased thickness of the epidermis) (Fig. 2. B).

Epidermis cells were composed of keratinocyte, melacocyte, langerhans cells and merkel cell. In order to produce and maintain the vital epidermal barrier, the keratinocyte, the main cell type in this tissue, undergoes proliferation and differentiation. During the progressive terminal maturation of the keratinocyte, its cellular morphology changes from typically cuboidal in the undifferentiated proliferative cells anchored on the epiderm-dermal junction in the basal layer, into a squamous morphology in the dead cells of the cornified layer. Between these layers, morphological changes mean taking shape of a prickle cell within the spinous layer and intracytoplasmic accumulation of dark structures, named keratohyalin granules, inside the granular layer, underlying the cornified barrier [4]. The typical epidermal organization into four layers reveals that inside the keratinocytes, the differentiation program is intended to produce the epidermal barrier. The appearance of different layers simply, called as acanthosis, results from progressive maturation of this cell type inside the epidermis.

In this study, it is apparent that the epidermis of dry skin induced by detergent has hyperplasia with abnormally accentuated keratinization and parakeratosis (open arrowhead). In addition, there is the infiltration of inflammatory cells (closed arrowhead) in the dermis. However, acanthosis was reversed by WET feeding and its application on dry skin. Group III, IV and V's epidermis were reversed most fully and showed no inflammatory cell in the dermis (Fig. 2. C, D and E). Thus, WET would recover the dry skin with

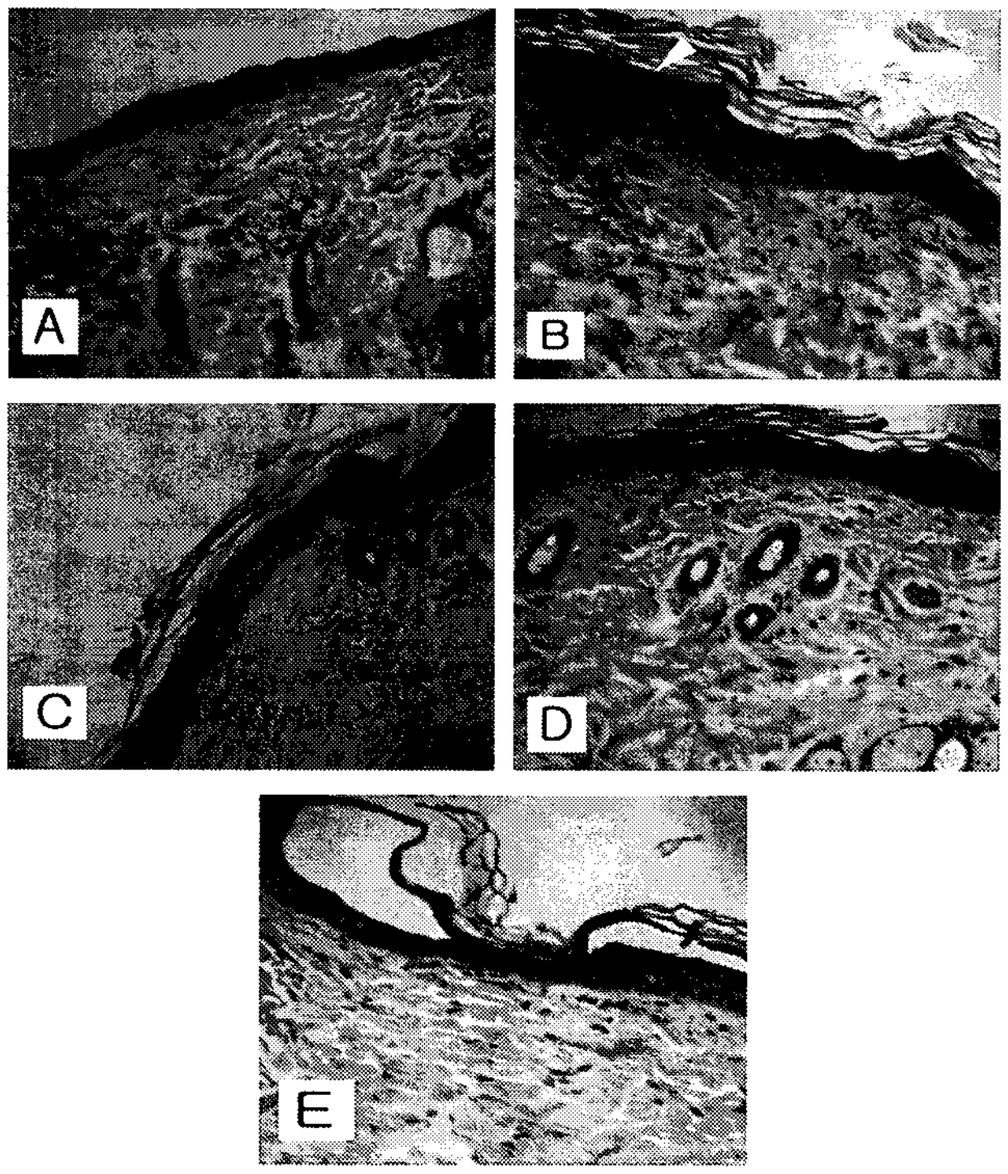

Fig. 2. Histological change of epidermis (×20). A: Skin from group I, B: Skin from group II, C: Skin from group III, D: Skin from group IV and E skin from group V.

abnormally accentuated keratinization and parakeratosis by preventing the inflammatory reaction and the infiltration of inflammatory cells on dry skin.

In general, the biochemical and physiological mechanisms for dry skin has been explained by the followings [1,7,14]. Dry skin is caused by two problems: (1) damage to the skin's protective barrier which produces excessive water loss through the skin, and (2) reduction in the con-

centrations of the skin's water-holding sugars and proteins: the proteoglycans and glycosaminoglycans. Proteoglycans are proteins linked to glucose chains called glycosaminoglycans and are vital components of connective tissues. They play an important role in modulating the structure and regulating the functions of the skin. In order to identify one of the biochemical mechanisms for the effects of WET on dry skin induced by detergent, protein analysis was carried out. All protein bands were shown at the range around the molecular size, 90,500~18,500 Da. And the protein band in the WET-treated groups showed the clearer band than that of group II. Especially, 115,000~462,000 Da proteins in the WET-treated groups were more clearly shown compared to that in group II, indicating that it would be the result form the regeneration of skin. The functional analysis of the deferentially expressed proteins will provide the valuable information for biochemical characteristics of the tomato extracts in recovering the damaged skin by detergent. These protein changes are especially associated with skin dryness induced by detergent. Steady and progressive loss of protein was greater in non-treatment group than in tomato treatment group. Thus, it seems that WET promote to synthesize protein lost in detergent-induced dry skin.

In conclusion, water-extracted tomato showed the decreased erythema, a marker of Inflammatory reaction, and reversed acanthosis induced by detergent. The amount of skin protein playing an important role in modulating the structure and regulating the functions of the skin was increased in groups treated and applied with water-extracted tomato compared to that in group with dry skin. Thus, one of reasons for the decreased erythema and acanthosis in dry skin induced by detergent would be explained by the increased amount of skin protein due to feeding and skin application of water-extracted tomato. However, it is not evident whether the increased amount of skin protein in groups treated and applied with water-extracted tomato are directly related with lycopene. Thus further study should be focused on the role of lycopene in protecting the epithelial tissue damaged by dry skin. However, water-extracted tomato is helpful for skin regeneration in dry skin and then would be recommended as a new solution for dry skin.

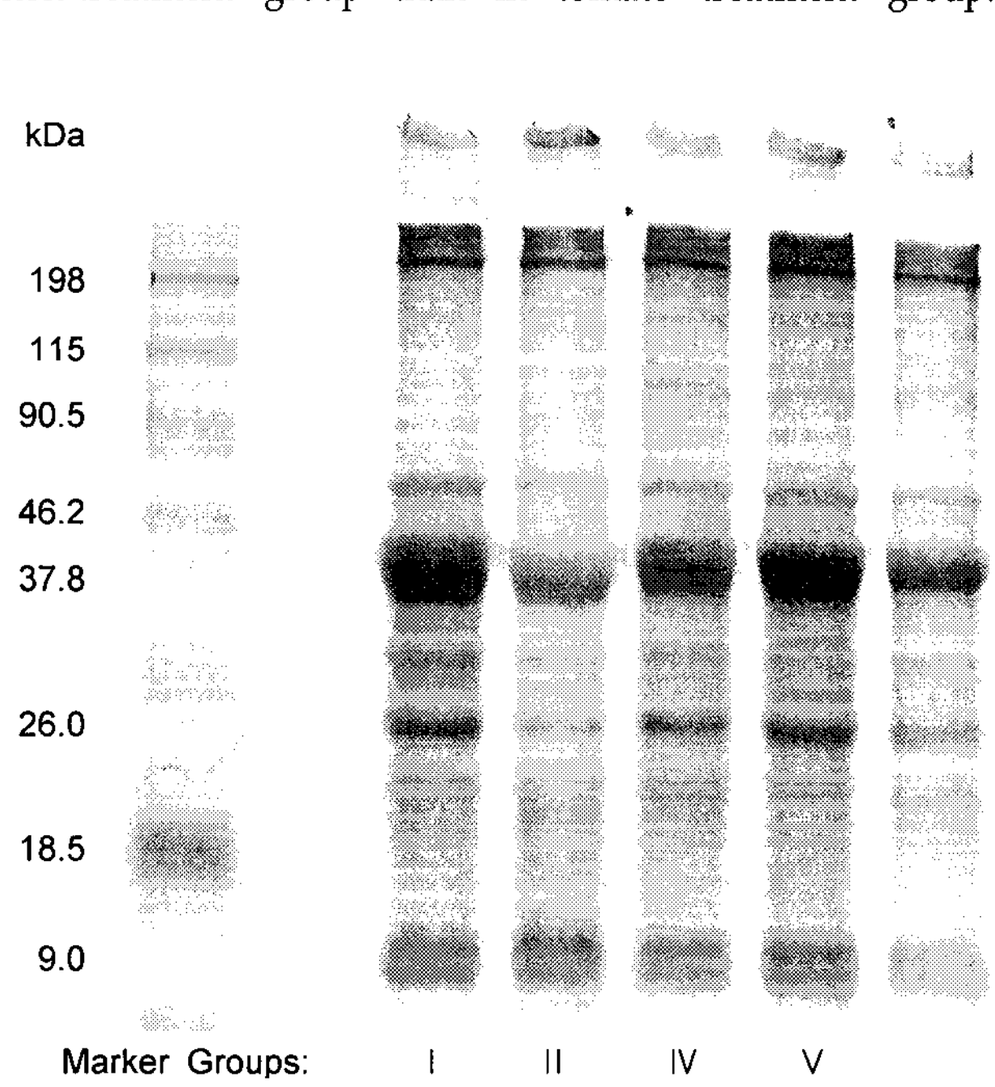

Fig. 3. Result of protein analysis from dry skin treated with WET and detergent: Proteins isolated from rat skins were separated by 12% poly-acrylamide gel. Marker protein (myosin: 198,000, ß-galactosidase: 115,000, Bovine serum albulmin: 90,500, glutamate dehydrogenase: 61,500, ovalbmin: 46,200, carbonic anhydrase: 37,800, myoglobin: 26,000, lysozyme: 18,500, aprotinin: 9,000 Da).

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초록 : 토마토추출물의 흰쥐 건성피부에 미치는 효과

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본 연구는 토마토 추출물의 주방세제 유도성 건성피부에 대한 효능을 실험쥐에 식이방법과 피부도포방법으로 처리하여 확인하였다. 약 3주간의 주방세제 처리에 의한 건성 증후는 홍반의 심각한 상태를 나타내는 4정도의 visual scoring이 모든 군에서 확인되었다. 그러나 약 1주간의 토마토추출물의 식이와 피부도포를 통해 상당히 완화되거나 정상 피부에 가까운 0.7-1.0 정도의 visual scoring이 확인되었다. 또한 광학현미경을 통해 건성의 또 다른 증후인 과각화 현상이 토마토추출물의 피부도포와 식이로 현저히 감소하는 것으로 확인되었다. 이러한 토마토추출물에 의한 건선성 증후의 완화는 피부 단백질 함량의 양적 변화를 유도하는 것으로 확인되어 토마토추출물의 건선성 피부의 예방과 개선에 주요한 기전으로 이해된다.