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TOPICAL GINSENG TREATMENT IN EXPERIMENTAL HYPERKERATOSIS

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ABSTRACT: *Effect of red ginseng treatment on experimentally induced hyperkeratosis was investigated by light microscopic observation, scanning electron microscope (SEM) examination,* epidermal enzyme activities and lipid contents. Both light microscopic observation and SEM examination showed that hexadecane induced epidermal hyperplasia, hypertrophy and hyperkeratosis by increasing the numbers as well as the sizes of epidermal cells including desquamating horny cells. The superficial horny cells were protruded around the base of hair shaft. Among red ginseng components, only saponin treatment inhibited epidermal hyperplasia and hyperkeratosis by reducing the thickness of epidermis and arranging the cornified cells. Saponin from Korean red ginseng inhibited abnormally increased epidermal LDH, ICD and G6PDH activities and reduced the contents of epidermal lipids induced by hexadecane. It seems that red ginseng saponin has preventive effect on experimental hyperkeratosis possibly by controlling the enzyme activities involved in epidermal cellular metabolism, resulting in reduced amounts of abnormal epidermal lipids.

Keywords: *Ginseng, Hyperkeratosis, Skin*

INTRODUCTION

Since ancient times ginseng has been used as a miraculous panacea for all kinds of illness and disease. Even though many research workers have tried to identify a scientific basis for the wide use of ginseng, ginseng still remains a mysterious herb but its value is becoming more recognized in the pharmaceutical and cosmetic fields. The cosmetic and therapeutic use of ginseng for skin was reviewed by Chang (1977); ginseng prevents or retards premature aging of skin and produces hyperemia therapy and perhaps countering effect on arteriosclerosis by enhancing circulation in the capillaries of the skin and increasing the supply for nutrients to the epidermis. Ginseng also reported to regulate dermal water balance by supplying trace amounts of minerals.

Non-toxic and non-irritating property of ginseng saponin to animal and human skin (Kim *et al.*, 1976; Kim and Woo, 1976; Lee and Kim, 1980) made ginseng possible for its use in skin-related studies. The first therapeutic use of ginseng for cutaneous disorder was performed by Russian scientists Igumnov and Burtkovski (1954). 10% alcohol extract of ginseng roots cured eczema, removing itching but treatment was not consistent. Later, Gredoire (1971) used ginseng root as a component of cold cream to control eczema and wrinkles. Furthermore, antiinflammatory effect of ginseng saponin ointment (Jun and Kim, 1982) and the effects of ginseng powder and ginseng sapoin on Candida albicans (Nam and Kim, 1982) were reported.

For cosmetic use of ginseng, Rovesti (1971) reported certain activating action of ginseng extract on sagging and wrinkled skin. Chang (1977) discussed some ginseng cosmetics such as bubble bath, lotion, cream, shampoo and femine hygiene liquids focusing on protection from radiation, chemical pollution and signs of aging. Topical application of ginseng extract or ginsenosides in the form of phospholipidic liposomes exerted favorable effects on aging skin, cutaneous elasticity, moisturizing activity on stratum corneum of the epidermis and circulatory derangements (Curri *et al.*, 1986; Gezzi *et al.*, 1986; Bombardelli *et al.*, 1988). Estrone, estradiol, and estriol were isolated from *Panax ginseng* root as cutaneous bioactivation components (Anguelakova, *et al.*, 1972) and use of topical ginseng appears to have an estrogen-like effect on genital tissues, contributing to postmenopausal bleeding (Hopkins *et al.*, 1988).

Our previous paper (Kim(Jun) *et al.*, 1989) reported that both topical application of red ginseng saponin and oral administration of 13-cis-retinoic acid had beneficial effects against experimentally induced hyperkeratinization. Present study was conducted to investigate the active components in Korean red ginseng which have preventive effects on hyperkeratosis through the *in vivo* study of franctionated red ginseng extract and to determine the mechanism of action of ginseng by the analysis of epidermal enzyme activities and lipid contents.

MATERIALS AND METHODS

Materials

n-Hexadecane, triethanolamine, hematoxylin, eosin, lactate dehydrogenase test kit, isocitrate dehydrogenase test kit, glucose-6-phosphate dehydrogenase test kit were obtained from Sigma Chemical Co. (St. Louis, MO). Paraplast tissue embedding medium was purchased from Monoject Co. (St. Louis, MO). Triglyceride test kit and total cholesterol test kit from Iatron Laboratories, Inc. (Tokyo, Japan) and non-esterified fatty acid test kit from Nissui Pharmaceutical Co. (Tokyo, Japan) were used. All other reagents were of guaranteed reagent grade commercially available.

Ginseng Sample Preparation

Ginseng saponin was prepared from water-saturated butanol fraction of powdered Korean red ginseng, followed by repeated filtration after addition of activated charcoal and methanol to obtain a pure yellow powder (Sanada *et al.*, 1974).

Water extract of Korean red ginseng was purchased from Korea Ginseng Factory, Korea Tobacco & Ginseng Cooperation. Ginseng sapoin solution (2% in 50% ethanol solution) was prepared for *in vivo* study. 28.6g of red ginseng extract was dissolved in 100 m*l* of vehicle (50% ethanol solution) because 28.6% extract contains approximately 2% saponin. Separately, red ginseng extract (28.6% in distilled water) was fractionated with ethyl ether and ethyl acetate to collect lipid-soluble (0.6%) and water-soluble substances such as carbohydrate, protein, sapoin and maillard reaction products (28%). Both lipid fraction and water soluble fraction were evaporated to dryness and dissolved in the vehicle for animal treatment.

Animal Treatment

Female Hartley guinea pigs, 450-500g from Sam Yuk Animal Breeding Lab. (Osan, Korea), were kept under conventional laboratory conditions with commercial laboratory chow (Jeil Animal Food Ind.), tap water and fresh vegetables ad libitum and used after 10 day of acclimation. Dorsal hairs, over an area of approximately 8 cm^2, were shaved with electric clipper 1 day prior to the experimet. 0.5 m*l* of each ginseng samples, ginseng saponin (2%), red ginseng extract (28.6%), lipid fraction (0.6%) and extract excluding lipids (28%), were topically applied to dorsal skin of each guinea pig 1 hr before the application of n-hexadecane (2 m*l*/kg B.W.) every day for 10 days. n-hexadecane was administered every other day during experimental period. 0.5 m*l* of 50% ethanol solution was topicaay applied daily as a vehicle. Animals were housed under subdued light and were killed by cervical dislocation at the 10th day of experiment. Excisional biopsies were taken from dorsal skins before and after sample treatment

Light Microscopy

Half of the biopsy material was fixed in 10% neutral buffered formalin and dehydrated with a graded series of alcohols before embedding in paraffin. Sections, 5 μ in thickness, were stained with hematoxylin and eosin and viewed under the light microscope. Microphotographs were taken with a Nikon Labphot Microscope (Tokyo, Japan).

Scanning Electron Microscopy (SEM)

The other half of the biopsy material served as a substrate for SEM. The specimens were fixed in 2.5% glutaraldehyde, followed by 1% osmium tetroxide. Following osmication, they were dehydrated through graded alcohols, transferred to isoamyl acetate, dried with a critical point dryer (Hitachi HCP-2), mounted on stubs, coated with gold in ion coater (Giko IB-3), and viewed through a Hitachi S-450 scanning electron microscope operated at 15 kV.

Epidermal Separation for Enzyme and Lipid Analysis

Two pieces (1.5 cm^2) of treated area of skin from each guinea pig were excised and immediately immersed in ice-cold water, placed in water bath (55°C, 30 sec), cooled in ice water and then blotted dry with filter paper. After stratum corneum of epidermis was removed with forceps, epidermis was scraped off from dermis with scalpel blade

on cold glass plate (Connor and Lowe, 1983). Epidermis was minced with scissors (< 1 mm^3), homogenized in 0.1M triethanolamine buffer (pH 7.6) and centrifuged (2,000 rpm, 5 min, 0-4°C). Soluble epidermal extract was collected for enzyme source and precipitate was dissolved, homogenized in the mixture of chloroform and methanol (2:1, v/v) filtered with glass fiber filter and dried under N_2 gas. Total lipid was measured by weighing method (Christie, 1982). Dried extract was re-dissolved in 1 m*l* of methanol and used for the analysis of triglyceride, total cholesterol and free fatty acid. Epidermal enzyme lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICD), glucose-6-phosphate dehydrogenase (G6PDH) activities and the contents of triglyceride, total cholesterol and free fatty acids were measured using commercial test kits. Protein concentration was determined by the method of Lowry *et al.*, (1951).

RESULTS AND DISCUSSION

Light Microscopic Observation

The skin is composed of an ectodermally derived, stratified squamous keratinized epithelium called the epidermis (E) and a mesodermally derived, dense irregular connective tissue called the dermis(D) (Johnson, 1984). Photo. 1 shows the dorsal skin of guinea pig before the treatment. The outmost cornified cells, called stratum corneum(sc), of the epidermis appear anucleated and filled with a fibrous material. With

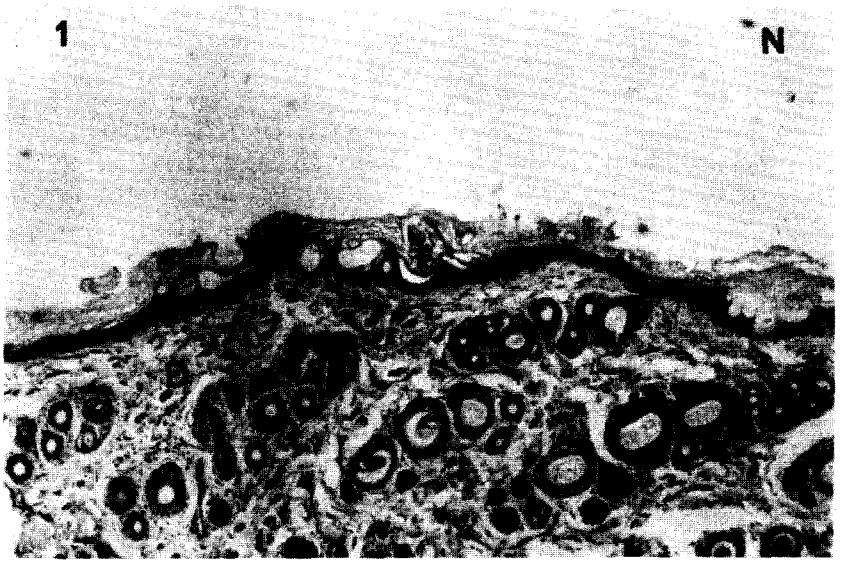


Photo. 1. Light micrograph of normal skin of guinea pig. The skin consists of two main layers, the surface epithelium, epidermis(E) and the subjacent connective tissue layer, dermis(D). The outer stratum corneum(sc) of epidermis is composed of dead, flattened cells which are constantly desquamating off the surface (×40).

Photo. 2. Whole skin of guinea pig treated with vehicle shows similar histological structure as normal skin (×40).

the light microscope, the stratum corneum appears as a poorly stained layer of dead and desiccated cells that can flake easily from the epidermis. Skins treated with 50% ethanol solution as a vehicle showed similar histological structure as normal skin (Photo. 2). Hexadecane (HD) induced epidermal hyperplasia, cellular hypertrophy and hyperkeratosis (Photo. 3). The epidermis is divided into four easily recognizable layers: stratum basale(sb), stratum spinosum(ss), stratum granulosum(sg) and stratum corneum(sc). Where the stratum corneum is particularly thick, there is a thin transitional zone between the stratum granulosum and the stratum corneum known as the stratum lucidum(sl). Hexadecane treated skin had stratum lucidum(sl, an arrow) which was found neither in normal nor in vehicle-treated skin. Hexadecane is reported to induce hyperplasia and hyperkeratinization to mammalian skin by increasing epidermal mitotic activity and provides a model for studying by keratinization process (Kirk and Hoekstra, 1964). Increased numbers as well as sizes of proliferative cells in stratum basale and stratum spinosum in Photo. 3 can be explainned by hexadecane-induced repeated mitosis. Abnormal type of keratin (nucleated, arrows) and increased layers of desquamating horny cells in stratum corneum were also shown. Topical application of red ginseng saponin (20 mg/kg BW) reduced the thickness of epidermis and the numbers of horny cell layers of stratum corneum induced by hexadecane (Photo. 4). However, the type of keratin was still laminated form (arrows) while normal skin has weave type of keratin as shown in Fig. 1 and Fig. 2. Treatment of red ginseng lipid fraction (Photo. 5), red ginseng extract excluding lipids (Photo. 6) and red ginseng extract with hexadecane demonstrated similar epidermal hyperplasia and hyperkeratosis as hexadecane-treated group (Photo. 3). Kirk and Hoekstra (1964) reported that

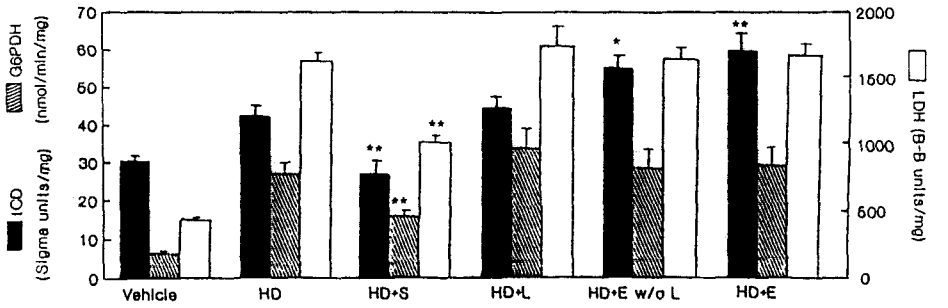


Fig. 1. Effects of red ginseng components on epidermal enzyme activities. Enzyme activities were measured in soluble epidermal extract from two pieces of dorsal skin areas (1.5 cm^2). Values are mean ± SE of 6 animals. An asterisk indicates values significantly different from hexadecane(HD) treated animals by student's t-test, *; $p<0.01$, **; $p<0.005$, S; saponin, L; lipid, E w/o L; extract excluding lipid, E; red ginseng extract.

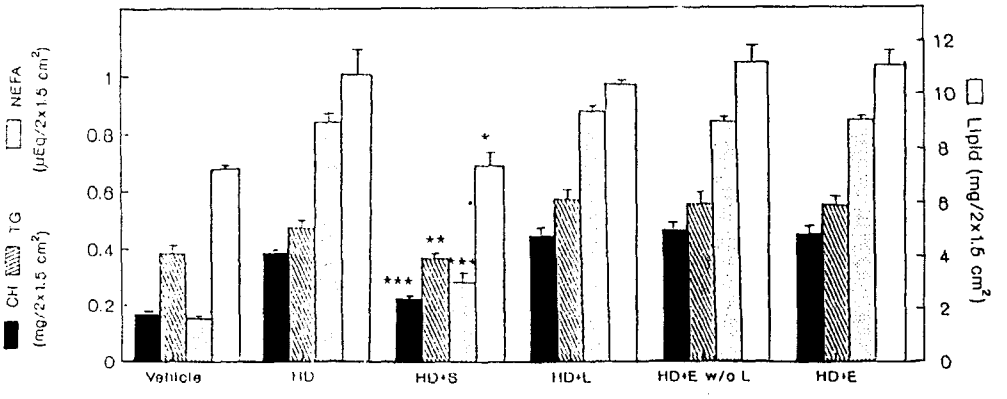


Fig. 2. Effects of red ginseng components on epidermal lipid contents. Lipid contents were determined in precipitates of epidermal homogenates from two pieces of dorsal skin areas (1.5 cm^2). Values are mean ± SE of 6 animals. An asterisk indicates values significantly different from hexadecane(HD) treated animals by student's t-test, *; $p<0.01$, **; $p<0.05$, ***; $p<0.005$. S; saponin, L; lipid, E w/o L; extract excluding lipid, E; red ginseng extract.

n-hexadecane induced a hyperkeratosis with epidemal proliferation in guinea pig skin which was similar to that seen in ichthyosis vulgaris in human. From this point of view, red ginseng saponin may be therapeutically valuable for some cutaneous disorders caused by epidermal hyperplasia.

SEM Examination

A high-power SEM view of the stratum corneum surface of hexadecane treated skin showed that many desquamating horny cells were protruded around the base of hair

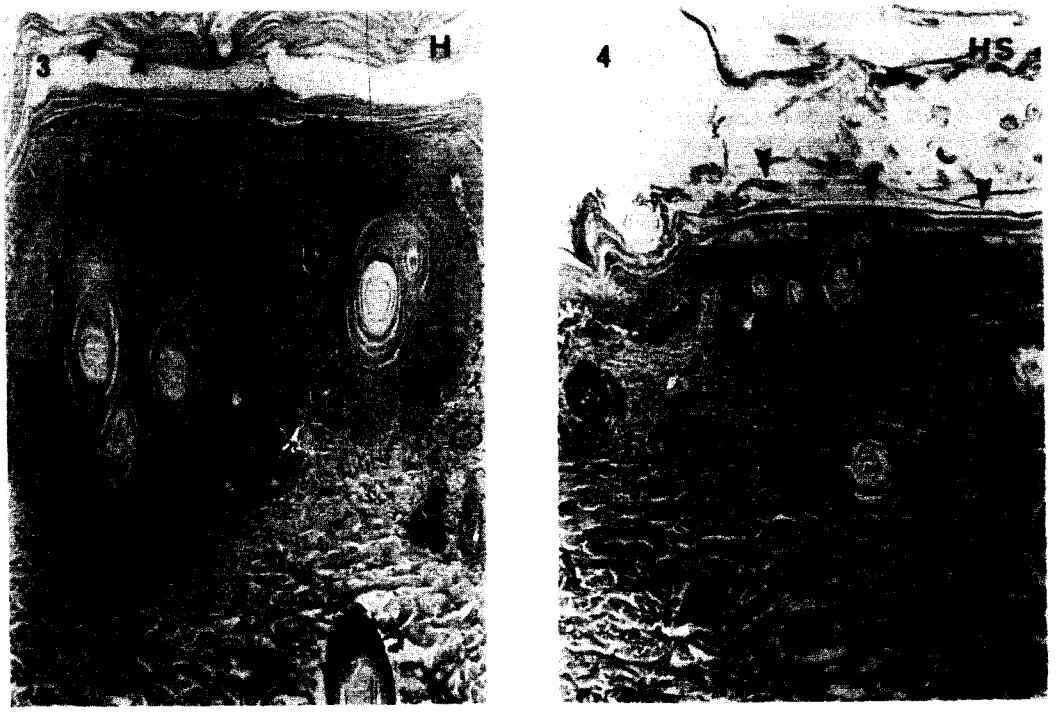


Photo. 3. Hexadecane treatment stimulates repeated mitosis in stratum basale (Sb) and daughter cells become converted to the dead cells of the stratum corneum (Sc), resulting in epidermal hyperplasia and hyperkeratosis. Abnormal type of keratin (anucleated, arrows) is shown (×40).
Photo. 4. Topical application of red ginseng saponin reduces the thickness of epidermis and the numbers of horny cell layers. Laminated type of keratin in stratum corneum (arrows) is still shown (×40).

shaft (Photo. 8). SEM of the acne of puberty shows comedones sitting within craters, protruding as large domed or conical structures (Papa, 1976). Insoluble cutting oil-induced comedones had chrysanthemum appearance. Lee *et al.*, (1986) reported that this difference of shape might be due to the absence of irritation and defatting effect of insoluble cutting oil. The superficial horny cells in the present study were somewhat different from already mentioned shapes. The reason might be explained by different hyperkeratosis-inducing agents. Treatment of red ginseng saponin gave less protruding horny cells and well-arranged stratum corneum surface was shown (Photo. 9). Flower like appearance of horny cells around hair shaft was demonstrated in red ginseng extract treated skin (Photo. 10).

Epidermal Enzyme Activities

Hexadecane treatment significantly increased epidermal enzyme activities such as lactate dehydrogenase (LDH), isocitrate dehydrogenase(ICD) and glucose-6-phosphate dehydrogenase (G6PDH) (Fig. 1). LDH is quite active in mammalian epider-

Photo. 5. Light micrograph of whole skin treated with red ginseng lipid fraction. Similar hyperkeratosis is seen as hexaadecane treated skin (×40).
Photo. 6. Whole skin of animals treated with red ginseng extract excluding lipids. No significant changes are shown (×40).

mis (Im and Adachi, 1966) and reflects epidermal metabolic activity. In contrast to most other tissues, epidermis converts most of the glucose to lactic acid even in the presence of oxygen(Gilbert, 1964). Halprin and Ohkawara (1966) compared LDH activity from different enzyme sources, epidermal sheets and epidermal homogenates and found that epidermal slice with its intact cellular organism had only about 1% activity of epidermal homogenates. They concluded that the cellular organization which limits LDH activity and thereby keeps useful concentration of pyruvate within the cell is obviously serving a necessary function. In this study, epidermal homogenate was used for relative comparison of LDH activities to determine the effects of ginseng samples on epidermal metabolic activity. ICD belongs to aerobic krebs cycle and is one of the sources of NADPH in the cell. Krebs cycle is highly active in skin as in other mammalian tissues (Cruiokshank *et al.*, 1958). Glucose-6-phosphate dehydrogenase is a key enzyme of the hexose monophosphate shunt pathway, in which ribose and NADPH are produced and used for cell metabolism. The activity of this enzyme is correlated to epidermal cell proliferation (Ohkawara, 1968). With this regards, hexadecane produced epidermal cell proliferation possibly by activating enzyme activities Among red ginseng samples, only saponin significantly prevented abnormally increas

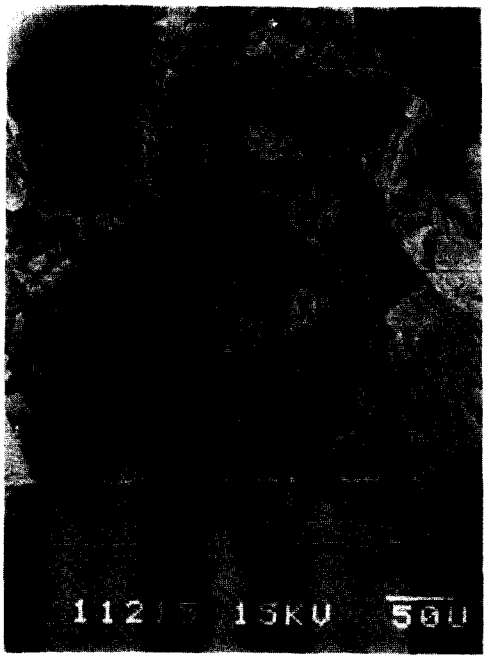
Photo 7. Treatment of red ginseng extract with hexadecane demonstrates hyperkeratosis which was shown in only hexadecane-treated animals (×40).
Photo. 8. SEM view of the stratum corneum surface of hexadecane-treated skin shows many desquamating horny cells protruded around the base of hair shaft (×260).

ed enzyme activities in epidermis while others had no effect. It is suggested that red ginseng saponin prevents experimentally-induced epidermal hyperplasia and hyperkeratosis by controlling activities of enzymes involved in epidermal cellular metabolism.

Epidermal Lipid Contents

Increasing number of acquired and genetic disorders of keratinization appear to be accompanied by lipid abnormalities and skin scaling abnormalities seem to be related to aberrant skin lipid metabolism(Elias, 1981). Most of skin lipids come from the sebaceous gland as sebum and remaining lipids come from the stratum corneum cells of the epidermis (Nicholaides, 1974). Epidermal lipids determined in present study were from epidermal cell because animals do not have sebaceous glands in human. Even through hamster flank organs and rat preputial glands have been used as animal models for human sebaceous glands, they are not comparable to sebaceous glands in many ways of function and metabolism(Madani *et al.*, 1985).

As shwon in Fig. 2, total lipid content in epidermis was significantly increased by hexadecane treatment. The order of increment was as follows: free faty acids (5.8



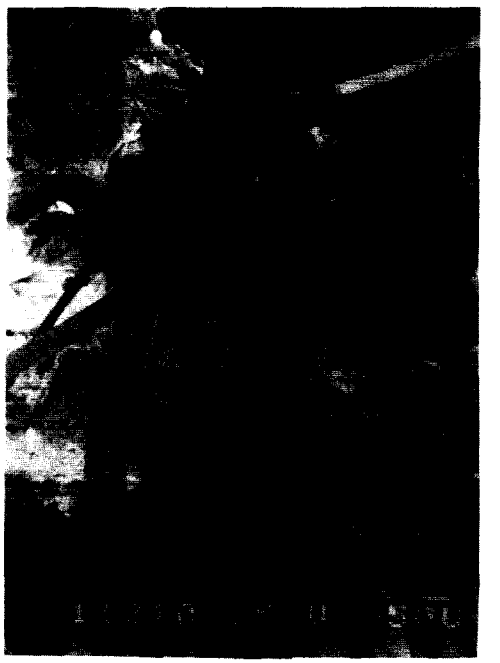


Photo. 9. The surface of dorsal skin treated with red ginseng saponin shows less protruding horny cells and well-arranged stratum corneum (×260).

Photo 10. Flower-like appearance of horny cells around hair shaft was demonstrated in the stratum corneum of guinea pig skin treated with red ginseng extract (×260).

times of vehicle value) cholesterol (2.5 times) and then triglyceride (1.3 times). Accumulation of lipids in epidermis might be caused by highly proliferative epidermal cells which are stimulated by hexadecane application. In late stage of keratinization, epidermal cells are somewhat removed from dermis, which is their source of nutrients, and the subcellular membranes begin to disintegrate. The biologically valuable fatty acids that make up these membranes are oxidized by the remaining mitochondria to yield the adenosine triphosphate required to complete keratinization process. The leftover fatty acids then make their small contribution to the surface lipid (Nicolaides, 1974). This report could explain large amounts of free fatty acids in hexadecane-treated epidermis *in vivo*. Topical application of red ginseng components showed similar effects on epidermal lipids as their effects on epidermal enzyme activities. Only ginseng saponin significantly reduced the contents of epidermal lipids induced by hexadecane; total lipid and triglyceride levels were similar to the levels before the treatment of hexadecane.

It is thought that red ginseng saponin has preventive effect on experimentally induced hyperkeratosis possibly by controlling the enzyme activities involved in cellular metabolism and resulting in reduced amounts of abnormal epidermal lipids.

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