International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 7,
2020 ISSN: 1475-7192

Effect of Polygoni Multifori Radix Extract on Menopause-Related Skin Aging

Eun-Suk Kim[1], Hye-Jin Kwon*[2]

[1] *Professor, Dept. Beauty Art, Seokyeong Univ., Sungbuk-gu Seoul Korea, 02713*

*[2]*Professor, Dept. Chemical Engineering, Soongsil Univ., Dongjak-gu Seoul Korea, 06978*

Poshop99@naver.com[1], kwonhj0070@ssu.ac.kr*[2]

Abstract

Background/Objectives: *The purpose of this study was to prepare an emulsion containing Polygoni Multifori Radix extract to help improve aging of skin menopausal women.*

Methods*: The ovaries of 6–8-week-old SKH-1 hairless mouse were resected to induce menopausal aging, and Polygoni Multifori Radix extract were applied. This experimental group was compared with normal and natural aging controls.*

Findings*: Histological observations of the epidermis demonstrated that the average thickness of epidermal lipid lamellae was 11.1%, the proportion of epidermal lipid lamellae was 5.73%, and the overall epidermal thickness was increased in the menopausal aging group. The observation of histological changes in the dermis also showed increased density of collagen and decreased amount of denatured elastic fibers.*

Improvements/Applications*: Thus, Polygoni Multifori Radix extract was found to reduce the damage in skin tissues caused by menopausal aging and to suppress the wrinkles.*

Keywords: *Polygoni MultiflorI Radix, menopausal women, collagen, elastic fibers, anti-wrinkle*

1. INTRODUCTION

Women's menopause, known as one of the aging phenomena, accounts for more than one-third of life in modern society with extended elderly life. The decline in ovarian function is caused by dysfunction of the gonadal axis leading from the hypothalamus, pituitary gland, and ovary, and this results in physical and mental changes such as alterations in sex hormone lipids and lipoproteins and bone metabolism [1]. In addition to aging, the number of tissue parenchymal cells decreases, resulting in the loss of most tissue weight in older age. This decrease in tissues leads to changes in collagen-constituting connective tissues such as bone, cartilage, and skin, and the formation of modified collagen and elastin by reduction of skin fibroblasts [2]. Menopausal aging caused by these factors has different characteristics from natural aging or photoaging. In particular, during the first 5 years of menopause, 30% of the collagen present in the skin is lost, causing rapid aging, and women appear to have wrinkles 3.5 times more severe than men [3].The skin contains keratinocytes, melanocytes, langerhans cells, fibroblasts, mast cells, etc. As these cells undergo aging, changes in their number and function make the skin look older and reduce physiological functions. Owing to the diminishing capacity of cell division, the thickness of the

epidermis decreases, the interface between the epidermis and the dermis becomes flat, and the area of contact decreases, resulting in blistering or peeling of the skin easily. Wrinkles and loss of elasticity reduce the thickness of the dermis and damage the skin shape owing to changes in the matrix protein of the dermis [4].

Polygoni multiflori radix contains anthraquinone derivatives and primarily comprises chryxophanol–emodin and others, including Rane–Physcion, chrysophanol, and lecithin[5]. It can be cultivated anywhere in Korea; it has been used for whitening symptoms of beard and hair, weakness in waist and knee, and severe pain in muscles and bones in the oriental medicine. Furthermore, it is known to be effective in uterine bleeding, hepatitis, skin bruise, nourishment tonic, detoxification, joint pain, blood pressure, arteriosclerosis, antioxidation, and strengthening immunity. Recently, with increasing research on various food supplements using Polygoni multiflori radix, there is a growing interest of the cosmetic field in it[6]. Through verification of skin pharmacological effects such as antioxidant whitening and antimicrobial effect, it was confirmed that Polygoni multiflori radix extract has an excellent antioxidant effect and is suitable for use as a cosmetic raw material for whitening, acne, and troubled skin[7].

The purpose of this study was to investigate the effects of Polygoni multiflori radix on menopause-related skin aging in artificially induced menopausal rats with low estrogen secretion. Polygoni multiflori radix, an edible plant, is expected to improve the symptoms of menopause-related skin aging.

2. Materials And Methods

2.1. *Preparation of emulsion containing Polygoni Multiflori Radix extract*

The Polygoni multiflori radix extract used in this experiment was purchased from a farm in Jeollanam-do, Korea. A total of 100 mL of distilled water was added to 10 g of dried Polygoni multiflori radix and extracted at 60°C–80°C for 24 h. The solids were filtered through a 200 mesh filter; only the extract was concentrated in a rotary vacuum concentrator and then completely dried and pulverized in a freeze dryer. The powder yield was approximately 12.5% compared with the raw materials. The emulsion utilized in the final experiment was used as o/w formulation containing 10% of the extracted Polygoni multiflori radix.

2.2. *Experimental animals*

Animals used in this study were 6-week-old female and male SKH-1 hairless mouse (OrientBio Co., Korea) and were approved by the Institutional Review Board of Chonbuk National University. All animals received feed and drinking water in a constant environment with temperature (22 ± 3°C), humidity (50 ± 12%), and a 12-h light cycle. In total, 70 mg/kg of ketamine (Yuhan Ketamine Inj., Yuhan Medica Co., Seoul, Korea) and 10 mg/kg of xylazine (Rompun inj., Bayer Health Care, LLC, Tarrytown, NY, USA) were intraperitoneally administered to anesthetize the rats that had been purified for a week to remove their ovaries. The surgical site of the experimental animal was sterilized with 10% betadine solution (Betadine Surgical Scrub, Korea Pharma, Seoul, Korea), and approximately 1.5 cm of skin below the dorsum, peritoneum, and abdominal muscles was dissected. The ovaries were exposed, the fallopian tubes were ligated with sutures, and the ovaries were excised. After ablation of both ovaries, the abdominal muscles, peritoneum, and skin were sutured with nylon yarn. Some animals were sutured without ovarian resection after opening and placed in normal control. The rats that underwent ovarian resection were used for the test after 2 weeks of recovery.

2.3. *Test group configuration and treatment*

The experimental group was divided into normal(N), natural aging(N-a), and menopausal aging groups(M-a), and six animals were allocated in each group. After the recovery period, the groups were separated to ensure that the mean weight and standard deviation among the groups were uniform. Once their entire skin were washed and dried completely for 10 min, 200 μL of samples were applied to the entire back twice a day for 10 days. To observe histological changes, tissue sections were prepared after staining with Van Gieson's and Verhoeff's stains and observed using a scanning electron microscope (stereo-scan-250-MK-III, UK). Tissue thickness, the number of inflammatory and mast cells, area of collagen and elastin, and distribution and area of lamellae were quantified by histomorphometric analysis using an image program.

2.4. *Data processing method*

Statistical analysis was performed using PASW Statistics 18.0 for windows (SPSS Inc., USA) to conduct one-way ANOVA and repeated measures ANOVA to confirm interactions between groups. A p value of 0.05 was considered statistically significant.

3. Results And Discussion

3.1. *Morphological observation of the skin surface*

After the sample was applied to the back of 8-week-old normal, aging, and menopausal aging groups for 10 days, the skin surface was observed using a scanning electron microscope, as shown in Figure 1. In the normal group, keratin that had not yet been eliminated remained in the pores, and the skin curves were uniform and lines were clearly formed. In the case of the natural aging group, the skin surface was smooth, but some keratin was separated and excised. In the case of the menopausal aging group, the keratin was cleaned up and the skin surface was smooth. Moreover, it can be observed that the wrinkles are alleviated in the menopausal aging group because the skin was less curved than that in the normal and natural aging groups. In other words, roughness was suppressed, and the surface became smooth. When visual differences were compared among the experimental groups, it was observed that the application of the sample was effective in improving the skin surface of the mouse in the menopausal aging group.

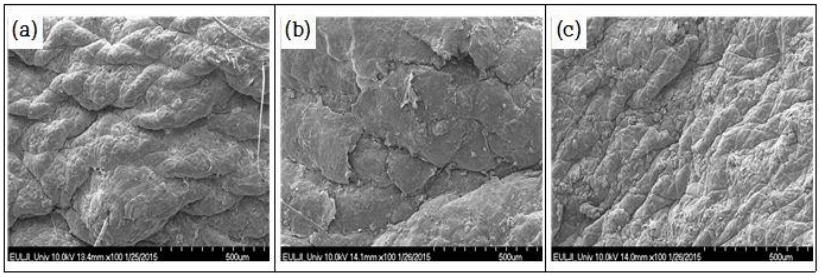

Figure 1. Scanning electron microphotographs of dorsal back skins surface of hairless mice(SKH-1) (x100)
(a:N, b:N-a, c:M-a)

3.2 *Histological observation of the epidermis*

Human skin constantly creates new epidermis through cell division and differentiation as it forms the interface with the external environment [8]. The normal epidermis of a healthy person acts as a defense against the escape of body fluids and the permeation of harmful substances, precisely controlling cell division, proliferation, differentiation, and elimination [9]. Therefore, proper thickness of the epidermis and stratum corneum lipid membrane of the stratum corneum are extremely important factors for maintaining young and healthy skin. Aging causes thinning of the dermis and epidermis and loss of the fat below these layers. The decrease in volume and overall effect of all three skin layers affects some important medical and cosmetic factors[10]. This indicates that the skin barrier function is impaired, and the key oil production such as sebum is reduced. Table 1 presents the changes in epidermal lipid atrophy of the three experimental groups. Examination of the average thickness of the epidermal lipid lamellae and the percentage of area occupied by the epidermis of the lipid lamellae shows that lipids in the stratum corneum of all three groups with the sample applied were maintained and improved. In particular, statistically significant changes were found in the menopausal aging group, which showed maximum atrophy, and the Polygoni multiflori radix extract was verified to help improve lipid distribution in menopausal aging. The average thickness of the epidermal lipid lamellae was changed by 4.62%, 8.48%, and 11.1%, and the ratios to the areas of the epidermal lipid lamellae were 1.85%, 4.57%, and 5.73%, respectively. Atrophy of the lipid layer causes not only dry skin but also various inflammatory diseases in old age. Thus, the efficacy of Polygoni multiflori radix extract is very significant.

Table 2 presents the overall change in thickness of the epidermis and Inflammatory Numbers. Compared with the thickness in the normal group, those in the natural aging and menopausal aging groups were relatively thin, but showed a statistically significant change because of the overall improvement after application of the sample. The thickness of the epidermis generally decreases with aging; however, in some cases, the thickness of the epidermis may increase owing to abnormal aging of photoaging or keratinization. Therefore, excessive thickening of the epidermis should also be carefully examined. The results of this study indicated a tendency similar to those noted in previous studies.

Table 1: Histomorphometrical Values Detected on the Hairless Mice Dorsal Back lipid barrier (a:N, b:N-a, c:M-a)

Groups	Controls			Test materials		
Histomorphometry	N	N-a	M-a	N	N-a,	M-a
Lipid barrier Lipid lamella thickness (μm)	14.34±1.40	4.39±1.19*	3.27±1.19*	15.27±2.25	8.97±1.88*	6.08±1.94*
Lipid lamella deposit (%/epithelium)	67.04±12.31	16.09±6.07*	12.12±2.24*	68.51±4.14	19.49±4.13*	31.85±4.58**

(p*<0.05, p**<0.01).

Table 2: Histomorphometrical Values Detected on Hairless Mice Dorsal Back Skins-General histology (a:N, b:N-a, c:M-a)

Groups	Controls			Test materials		
Histomorphometry	N	N-a	M-a	N	N-a,	M-a
General histology Epithelial (μm)	22.46±4.16	12.34±6.12 *	10.23±2.2*	26.72±2.88	22.57±2.70 *	18.59±3.12*
Inflammatory Numbers (cells/ mm2)	42.50±18.88	96.09±7.08 *	654.02±9.14 *	50.83±11.23	81.33±11.57	274.50±26.70 *

(p*<0.05)

3.3. *Histological observation of the dermis*

The main feature of menopausal aging is the change in the dermal layer, particularly collagen fibers. After menopause, ovarian function decreases, leading to a decrease in blood levels of estrone and estradiol; particularly, estradiol levels are significantly reduced [11]. Collagen decreases owing to hormonal change, and skin wrinkles increase. In natural skin aging, collagen decreases by 1% per year, whereas in menopausal skin aging, collagen diminishes by an average of 2.1%. The rate of collagen synthesis swiftly declines, and the skin looks rapidly old in the first 5 years after menopause [12]. Although the physiological aging that occurs throughout the body reduces skin thickness and collagen synthesis, a decrease in estrogen accelerates this phenomenon in menopause, which makes the skin rougher and drier and leads to a decrease in elasticity.

The amount and morphology of collagen in the dermis was observed [Figure 2]. In the normal group, collagen density was dense and the arrangement was regular, whereas in the natural and menopausal aging groups, collagens were destroyed and the thickness of the dermis slightly increased in the process of healing and proliferation. During the proliferation after healing, the arrangement was irregular and some denatured collagen was also observed. Even after sample treatment, the difference among the groups was confirmed. In the menopausal aging group, the change in density and arrangement was most remarkable after the sample treatment, which demonstrated its efficacy.

The change and disappearance of elastic fibers in the dermis showed more significant differences than those of collagen among the groups [Figure 3]. The elastic fibers were regular in the normal group, whereas the dermis became thick and denatured and tangled fibers increased in the aging group. After sample processing, the aging group displayed a pattern that was similar to that in the normal group. In particular, elastic fiber degeneration is the biggest feature of photoaging and shows abnormal overgrowth [13]. This phenomenon was partially observed in the natural aging and menopausal aging groups and gradually decreased after sample treatment. Elastic fibers are composed of amorphous elastic fibers and microfibers. In photoaged skin, it is reported that the deformation occurs because the fine elastic fibers present vertically underneath the epidermis gradually break down and

disappear with the accumulation of elastic fibrosis substances in the upper dermis, and the fine elastic fibers that are horizontally arranged increase in number [14]. This histological observation confirmed that the treatment with the sample reduces the damage to the dermis.

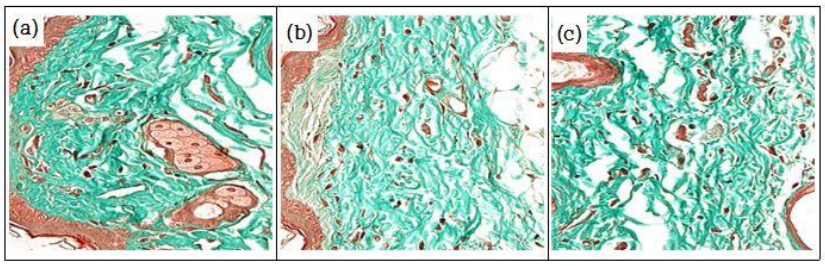

Figure 2. Photographs of histological sections of hairless mice(SKH-1) dorsal back skins by Masson trichrome staining (Original magnification: 200x, Arrow: Collagen Fiber). (scale bar : 100μm) (a:N, b:N-a, c:M-a)

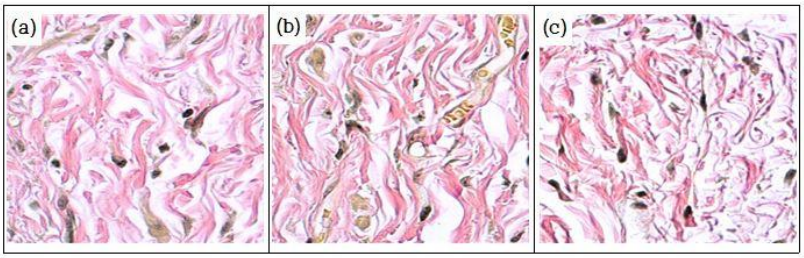

Figure 3. Photographs of histological sections of hairless mice (SKH-1) dorsal back skins by Verhoeff-van Gieson staining (Original magnification: 400x, Arrow: Elastin fiber). (scale bar : 100μm) (a:N, b:N-a, c:M-a)

3.4. *Changes in inflammatory response of skin tissue*

The distribution of inflammatory cells and mast cells in the dermis was observed. The normal group had few inflammatory cells and mast cells, whereas the aging group had several inflammatory and mast cells. In all three groups, the mast cells were reduced after sample treatment, particularly in the menopausal aging group. Changes in the inflammatory response are presented in Figure 4. As we get older, the number of mast cells in our skin decreases, but UV rays increase the number of mast cells in our skin. In particular, more mast cells can be observed in exposed areas [15]. These findings were also verified in previous studies.

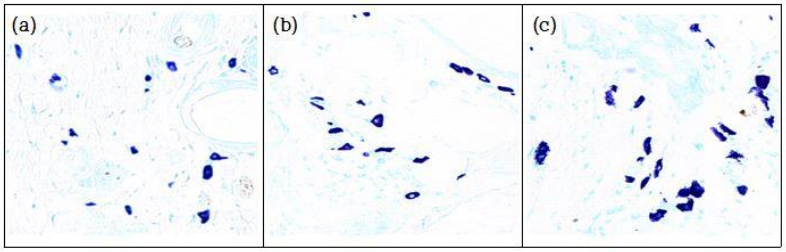

Figure 4. Photograpfs of histological sections of hairless mice (SKH-1) dorsal abck skins by Toluidine blue staining (Original magnification: 100x, Arrow: Mast cell). (scale bar : 100μm) (a:N, b:N-a, c:M-a)

4. CONCLUSION

Polygoni multiflori radix, known as a dietary supplement, contains high amounts of anthraquinone derivatives and lecithin, which may improve chronic symptoms in menopause.

The purpose of this study was to evaluate the effects of Polygoni multiflori radix extract on improvement of menopause-related skin aging in ovariectomized rats to induce menopause. Histological observation of the epidermis verified its effects and showed that the average thickness of epidermal lipid lamellae was 11.1% and the ratio of epidermal lipid lamellae was 5.73% in the menopausal aging group. The observation results of the histological changes of the dermis confirmed that Polygoni multiflori radix extract is effective for wrinkle improvement, which is a representative sign of skin aging. This indicates that Polygoni multiflori radix extract is a functional ingredient that can help prevent and improve skin aging in menopause.

ACKNOWLEDGMENT

This research was supported by 2018 Basic Science Research Program through the National Research Foundation of Korea funded by the Education, Science and Technology. (No.2018008274)

REFERENCES

[1] Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. The Journal of the American Medical Association. 1998 Oct;280(7):605-613.

[2] Liu J, Burdette JE, Xu H, Gu C, Breemen RV, Bhat KP, et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. Journal of agricultural and food chemistry. 2001 Sep;49(5);2472-2481.

[3] Mcconkey B, Fraser GM, Bligh AS, Whiteley H. Transparent skin and osteoporosis. Crossref. 1963 Mar;281(7283):693-695.

[4] Desmond JT. Introduction to skin aging. Journal of Tissue Viability. 2017 Jan;26(1):37-46.

[5] Kim MS. The Phytoestrogenic Effect of Daidzein in Human Dermal Fibroblasts. J Soc Cosmet Scientists Korea. 2014 Sep;40(3):279-287.

[6] Kim KH, Youn CK, Kim IJ, Lee KJ, Kim YH, Hong ST, et al. Morphological and Cytogenetic Analysis of Colchicine-induced Tetraploids of Fallopia multiflolra Haraldson. Korean J. Medicinal Crop Sci. 2018 Oct;26(5):362-369.

[7] Oh JS, Hong JH, Park TY, Yun KW, Kang KY, Jin SW, et al. Chemical Constituents in Polygonum multiflorum Thunberg Root Based on Various Dry Methods. Korean J Plant Res. 2018 Dec;31(4):283-293.

[8] Waller JM, Maibach HI. Age and skin structure and function, a quantitative approach blood flow, pH, thickness, and ultrasound echogenicity. Skin Res Technol. 2005 Dec;11(4):221-235.

[9] Ghadially R, Brown BE, Sequeira SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. J Clin Invest. 1995 Oct;95(5):2281-2290.

[10] Scharffetter K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, et al. Photoaging of the skin from phenotype to mechanisms. Exp Gerontol 2000 Sep;35(3):307-316.

[11] Lee SJ, Kwon GT, Choi HR, Gim SW, Choi DH, Kong HS, et al. Establishment of the Male Menopause Animal Model Using Rats. Journal of Agriculture & Life Science. 2018 OCT;52(5):91-100.

[12] Gail J. Molecular mechanisms of skin ageing. Mechanisms of Ageing and Development. 2002 Oct;123(7):801–810.

[13] Fisher GJ, Choi HC, Bata CZ, Shao Y, Datta S, Wang ZQ, et al. Ultraviolet irradiation increases matrix metalloproteinase-8 protein in human skin in vivo. J Invest Dermatol. 2001 Apr;117(2):219–226.

[14] Francis C, Robert L. Elastin and elastic fibres in normal and pathologic skin. Int J Dermatol. 1984 Mar;23(3):166–179.

[15] Kohen R, Gati I. Skin low molecular weight antioxidants and their role in aging and in oxidative stress. Toxicology. 2000 Sep;148(2):159–167.