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THE ROLE OF ANTHOCYANINS IN ESTROGEN-DEFICIENT SKIN

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Abstract:
Decreased levels of estrogen hormone in women, particularly during menopause, causes skin aging, a condition referred to as estrogen-deficient skin (EDS). This condition is characterized by the loss of collagen, elastin, and fibroblast function, increased vascularization, and activity of matrix metalloproteinase (MMP) enzyme, which contribute to aging. This study aims to evaluate the potential of anthocyanins to mitigate skin aging due to estrogen-deficient skin. Skin aging is caused by a combination of intrinsic and extrinsic factors. One of the intrinsic factors is the decrease in the levels of estrogen hormone in menopausal women, resulting in EDS. This condition leads to increased production of free radicals, cellular damage, decreased synthesis of the extracellular matrix (ECM), and elevated activity of collagen-degrading enzymes. Anthocyanin is a source of antioxidants that have estrogenic properties and function as a selective estrogen receptor modulator (SERM) capable of attaching to estrogen receptors and preventing skin aging. Conclusion: The pathophysiological mechanism of skin aging due to decreased levels of estrogen hormone can be inhibited by administering anthocyanins which act as a SERM to prevent skin aging due to EDS.

Keywords: *skin aging, estrogen, estrogen-deficient skin, anthocyanin, SERM*

1. Introduction:

The skin is the outermost and largest organ of the human body and protects the muscles and internal organs. As the number of elderly populations in the world increases, the problem of aging, particularly skin aging, is becoming more prevalent. Skin aging refers to the progressive decline in the function and capacity of the skin, caused by intrinsic and extrinsic factors. Intrinsic factors include genetics, cell metabolism, and hormones, while extrinsic factors include ultraviolet and infrared radiation, as well as environmental carcinogens such as air pollution. The accumulation of all factors gradually changes the structure and function of each skin layer, causing changes in the appearance of the skin. Intrinsic aging impacts skin morphology and physiology, resulting in dryness, wrinkles, sagging, and slow wound healing, which is an inevitable process. In contrast, extrinsic aging impacts skin elasticity, resulting in the formation of deep wrinkles, a rough skin surface, and the development of benign tumors and precancerous lesions. Skin aging due to hormonal decline in women is primarily caused by menopause.

Menopause is characterized by the permanent cessation of menstruation (amenorrhea) for a year or more, caused by the cessation of ovarian follicle activity and a subsequent decrease in the production of the estrogen hormone. As of 2021, 26% of the global female population was aged 50 years or older, indicating that most women will spend at least a quarter to a third of their lives in a menopausal stat.[1] Estrogen hormone plays a significant role in modulating skin physiology, targeting keratinocytes, fibroblasts, melanocytes, hair follicles, and oil glands, as well as enhancing angiogenesis, wound healing, and immune response. Additionally, estrogen hormone regulates the metabolism of extracellular matrix (ECM) components, so that a decrease in estrogen levels during menopause causes decreased levels of ECM.[2]

Changes in skin due to estrogen deficiency, known as estrogen-deficient skin (EDS), include the loss of collagen, elastin, and fibroblast function, vascularization, and increased activity of matrix metalloproteinases (MMPs) enzyme. These changes lead to cellular and extracellular degradation, resulting in dry, scaly skin, wrinkles, atrophy, impaired wound healing, reduced antioxidant capacity, decreased attractiveness and compromised psychological well-being, all of which contribute to skin aging. EDS affects all layers of the skin, manifesting in both structural and functional changes.[3]

Skin aging during menopause caused by decreased estrogen levels or EDS can inhibited by administering hormone replacement therapy (HRT) in the form of estrogen.[4] Although systemic HRT can improve the signs of EDS, including skin dryness and atrophy of the skin and vagina, it carries various risks, such as endometrial hyperplasia or cancer, and concerns about an increased risk of breast and ovarian cancer, which limit its use to treat skin disorders. As a result, alternative medicine and topical therapy are conducted to improve skin changes due to menopause and maintain youthful skin.[5]

One alternative therapy that is currently being developed to prevent and treat menopausal symptoms is phytoestrogen.[6] Phytoestrogens are chemical compounds originating from plants with estrogenic effects in animals. One of the phytoestrogens that is widely studied is the flavonoid group. Flavonoids have a similar structure to estrogen, allowing them to attach to and activate estrogen receptors on target cells. Several phytoestrogens have been identified as binding to both types of estrogen receptors, ERα and ERβ, inducing the expression of estrogen-responsive genes and triggering cell proliferation. Phytoestrogens are classified as one of the selective estrogen receptor modulators (SERMs) because of their ability to attach to receptors and exert estrogen-

like effects.[7] Anthocyanins are flavonoids whose structure similar to flavanones and isoflavones and have been proven to exhibit estrogenic activity both in vitro and in vivo.[8]

2. Material and Method

The type of research used is descriptive research. To evaluate the potential of anthocyanins to mitigate skin aging due to estrogen-deficient skin.

3. Result

Menopause and Skin Aging

Menopause typically occurs between the ages of 45 to 55 years, with an average age around 50 years in Western populations and earlier in women from developing countries. The number of menopausal populations in the world continues to increase. The current increase in life expectancy has resulted in an increase in the number of elderly people in the population.[9] Because women generally have a longer life expectancy than men and the age at which menopause occurs tends to remain constant, the number of postmenopausal women in society is increasing.[10]

Estrogen is the primary sex hormone in women, responsible for regulating the function of female reproductive system, as well as the development of secondary sexual characteristics that appear during puberty and sexual maturity. Estrogen exerts its effects by binding to a specific receptor, known as the estrogen receptors (ERs), to activate transcription processes and/or signaling events that result in the control of gene expression, mediated by direct binding of the ER complex to specific sequences in the gene promoter (genomic effects), or by mechanisms which do not involve direct binding to DNA (non-genomic effects).[11] The biological functions of estrogen are modulated by two types of ERs, namely ERα and ERβ. These receptors, which have similar affinities for estrogen, are often found in the skin[10], with ERβ being more widely distributed than ERα.[12] Expression levels of ERα and ERβ decrease from the perimenopausal years onwards as women enter the state of estrogen deficiency.[13]

A decrease in estrogen levels triggers an increase in the activity of MMPs enzyme, causing a decrease in collagen production.[14] Extracellular matrix proteins such as collagen and elastin are degraded by MMPs, while their activity is inhibited by tissue inhibitors of metalloproteinases (TIMPs).[15]

Estrogen can regulate the expression of insulin-like growth factor-1 (IGF-1) molecule secreted by dermal fibroblasts which supports the proliferation of keratinocytes in the epidermis. IGF-1 is a powerful growth factor that promotes the proliferation and survival of almost all cell types.[16] The downregulation of ERβ, as well as estrogen inhibitors, attenuates the stimulatory effect of estrogen on IGF-1 receptor expression.[17] Additionally, estrogen functions as an antioxidant because it enhances the regulation of antioxidant enzyme expression through intracellular signaling pathways, inhibits oxidative damage, and offers protection against degenerative diseases.[18] Estrogen reduces oxidative stress by preventing the formation of reactive oxygen species (ROS) and counteracting free radicals. It can also upregulate endogenous antioxidant defenses through indirect mechanisms on the expression and activity of enzymes such as SOD, GPx1, and GPx4.[19]

Aging of the epidermis is characterized a reduced capacity for barrier function and recovery after damage. The thickness of the epidermis decreases, and the contact surface area between the dermis and epidermis is reduced, leading to a smaller exchange surface for nutrient supply to the epidermis and a weakened proliferation ability of basal cells. The epidermal-dermal junction and the dermis also become thinner. Flattening of the epidermal-dermal junction results in fewer cells, nutrients, and oxygen, contributing to the formation of wrinkles. The extracellular matrix in the dermis undergoes both intrinsic and extrinsic structural and functional changes during skin aging, including changes in type I and type III collagen and collagen ratio, impaired synthesis of the ECM molecules, and changes in elastic fibers.[20] Collagen is a fibrous protein, the main constituent of the dermis, and combined with elastin, maintains skin elasticity and flexibility. Collagen and elastin decrease during menopause, causing the dermis to sag and the surface of the skin to wrinkle and sag. The most abundant collagen types in the skin are type I (80%) and type III (15%).[10] Collagen, elastin, and other ECM components are synthesized by fibroblast cells. [21]

Estrogen-Deficient Skin

The skin is the largest organ in the human body. Its total weight ranges between 2.7 and 3.6 kg and receives one-third of the body's blood supply. The thickness of the skin varies from 0.5 to 6.0 mm and consists of cells and extracellular matrix. The skin structure consists of three layers: the epidermis, the outermost and thinnest layer; the dermis, a thicker layer located beneath the epidermis; and the hypodermis (subcutaneous fat tissue) beneath the dermis, which is composed of loose connective tissue.[22]

A decrease in estrogen levels is associated with deteriorating skin health and function that negatively impacts dermal cellular and homeostatic mechanisms, as well as other important biological functions. Changes in the skin that is deficient in estrogen or estrogen-deficient skin (EDS) include a loss of collagen, elastin, and fibroblast function, vascularization, and increased activity of MMPs enzymes. These changes result in cellular and extracellular degradation which causes dry, scaly skin, wrinkles, atrophy, impaired wound healing, reduced antioxidant capacity, decreased attractiveness, and compromised psychological well-being, which contribute to skin aging.[10, 23]

Skin aging results from a combination of chronological, environmental, genetic, and hormonal factors. Because low estrogen levels and their associated skin manifestations can be due to iatrogenic causes and because the term “menopause” does not capture the broad age range of women who experience these symptoms, the term EDS is used. Estrogen is a steroid hormone which is synthesized from cholesterol in the ovaries in premenopausal women and in peripheral tissues in postmenopausal women.[10]

Both ERα and ERβ estrogen receptors have been identified in the skin. Histologically, the expression levels of these receptors begin to decline from the perimenopausal years onwards as women enter a state of estrogen deficiency. Although both ERs have similar affinities to estrogen, their expression profiles are tissue-specific, with ERβ being more widely distributed in the skin than ERα.[12]

Estrogen helps maintain and restore skin moisture by increasing sebum secretion, primarily by regulating insulin-like growth factor receptor expression and promoting growth factor production by fibroblasts, which in turn induces lipogenesis and maintains skin moisture. The progressive thinning of the skin in EDS is largely due to the loss of collagen and decreased collagen deposition. The most levels of collagen in the skin, particularly type I (80%) and type III (15%),

correlate with estrogen levels. Supplementation with estrogen hormone replacement therapy both systemically and topically in EDS has been shown to increase collagen synthesis.[10]

Research shows that estrogen deficiency accelerates the loss of skin elasticity by triggering ultrastructural changes in elastin fibers, leading to the formation of wrinkles. Skin thickness typically increases until it reaches a peak at the ages of 35 and 49 years, and then begins to decline with age.[24] Research shows that skin collagen decreases by up to 30% in the first five years after menopause, collagen decreases by around 2.1% per year, and skin thickness decreases by 1.1% per year.[10] The decrease in collagen, water content, and glycosaminoglycan (GAG) content causes a thinning effect on the skin, with osteoporosis being associated with skin thinning. Histological evidence demonstrates decreased skin thickness in postmenopausal women, with the epidermis showing thinning and the disappearance of rete ridges. Several studies have shown that estrogen supplementation increases skin thickness.[24]

Additionally, experimental data suggest that the presence of estrogen may protect skin cells from oxidative damage, and the dramatic decline in estrogen levels during menopause may cause the skin more susceptible to oxidative damage. The decrease in estrogen levels during menopause leads to changes in skin characteristics, including loss of collagen, elastin, and estrogenic function, as well as vascularization and increased activity of MMP enzyme. These changes contribute to cellular and extracellular degradation leading to dryness, wrinkles, atrophy, impaired wound healing and barrier function, reduced antioxidant capacity, particularly in defense against reactive oxygen species (ROS) and oxidative stress, decreased attractiveness, and compromised psychological well-being, which cause aging.[25]

As estrogen levels decrease with age, collagen production is disrupted, whereas the production of various MMPs, especially MMP-1, MMP-2, MMP-3 and MMP-9, increases. The production of all these MMPs, except MMP-2, is regulated by activator protein-1 (AP-1), a transcription factor that inhibits procollagen gene expression in fibroblasts and stimulates MMP gene transcription in fibroblasts and keratinocytes. AP-1 expression is higher than normal in aged fibroblasts from skin samples in vitro and is also higher in older skin in vivo.[15]

Antioxidant defenses decrease with age, leading to the accumulation of oxidative damage over the years. Free radicals and ROS from aerobic metabolism initiate the aging process.[26] Increased ROS activate various pathways, including the mitogen-activated protein kinase (MAPK) which induces AP-1 and consequently increases the expression of MMPs, resulting in reduced collagen levels in aged skin.[15] Menopause causes increased levels of the cytokine tumor necrosis factor-α (TNF-α), which increases collagenase synthesis and inhibits collagen formation. TNF-α stimulates the expression of the MMP-9 gene, an enzyme that contributes to skin aging through skin damage. Persistent exposure of epidermal cells to TNF-α impairs MMP-9 production and results in permanent damage to the epidermis. Inflammation further upregulates MMP-9, with its gene transcription being regulated by the transcription factors AP-1 and nuclear factor kappa B (NF-κB). TNF-α increases the binding activity of these transcription factors to the MMP-9 deoxyribonucleic acid (DNA) sequence, thereby increase in MMP-9 protein production. As a result, cosmetic and pharmacological products that can inhibit the MMP-9 transcription factors may help skin renewal.[27]

Collagen in the dermis is relatively stable, synthesized from procollagen with the help of specific enzymes and degraded by collagenase. Research in mice has shown that estrogen inhibits collagen degradation. There is a complex relationship between regulatory factors, receptors, and enzymes in maintaining collagen balance. Decreased collagen in the dermis plays a significant role

in the pathogenesis of skin atrophy, and a strong relationship has been found between decreased collagen and estrogen deficiency during menopause.[24]

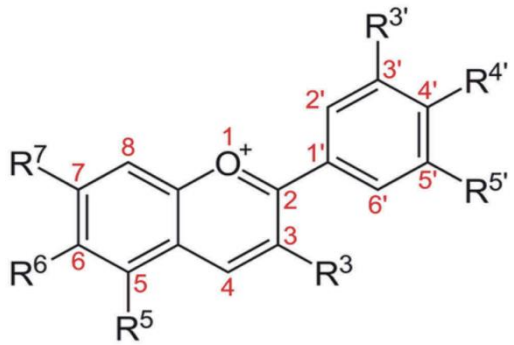
Hormone Replacement Therapy

To treat skin disorders such as thinning that occurs due to EDS, estrogen hormone replacement therapy (HRT) is used. HRT is available in systemic, transdermal, and topical forms.[28] Estrogen HRT has been proven to be effective in reducing menopausal symptoms such as the frequency and intensity of hot flushes, sleep disturbances, and the risk of bone fractures.[29] However, it is associated with an increased risk of endometrial and ovarian cancer[30], necessitating the exploration of other alternative therapies that are safer, namely phytoestrogens, which are compounds derived from plants with estrogen-like properties.[31]

Phytoestrogens have a similar structure to estrogen, can attach to estrogen receptors, and are one of the SERM. As SERMs, phytoestrogens can bind to ERα or ERβ although their affinity for estrogen receptors is dose-dependent and generally lower than endogenous estrogen. Genistein, one of the examples of phytoestrogens, has been shown to be able to attach to both estrogen receptors but exhibits a stronger affinity for ERβ compared ERα[7] and acts as a natural SERM.[32] The mechanism of action of phytoestrogens is similar to estrogen: they bind to estrogen receptors, and the ligand-receptor complex induces the expression of estrogen-responsive (ERE) genes. Several studies show that the use of phytoestrogens is safe and does not significantly increase endogenous estrogen levels. Until now, there have been no reports linking phytoestrogen as HRT with an increased risk of vascular disorders and breast cancer, making them a safe substitute for estrogen HRT.[2]

Anthocyanins as Phytoestrogens to Treat Estrogen Deficient Skin

Anthocyanins are a major subgroup of flavonoids, which are water-soluble plant pigments responsible for the blue, purple, and red colors in many plant tissues. The anthocyanin molecule consists of anthocyanin aglycone and several sugar groups.[33] At an acidic pH, anthocyanins exhibit a positive charge and exist in equilibrium among four molecules, namely flavylium cation, quinoidal base, hemiacetal base, and chalcone. The relative amounts of these four molecular forms vary depending on the pH and type of anthocyanin. This structural uniqueness is one of the key factors influencing the absorption, metabolism, bioavailability, and biological activity of anthocyanins.[34]



Picture 1. Basic structure of anthocyanin[35]

Anthocyanins are a subclass of phenolic phytochemicals. In their glycosylated form, they are referred to as anthocyanins, while their aglycone counterparts are known as anthocyanidins.

The conjugation of anthocyanins produces red, blue, and purple colors in plants. Anthocyanidins are categorized into 3-hydroxyanthocyanidins, 3-deoxyanthocyanidins, and O-methylated anthocyanidins. The most common types of anthocyanidins are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin.[35]

Anthocyanins are absorbed in the intestine through passive diffusion, which is different from other types of flavonoids which are not easily absorbed by this mechanism. Various molecular sizes and types of sugar or acylated groups in anthocyanins affect their absorption. For example, anthocyanins in purple sweet potato tubers can be detected intact in plasma and urine of humans and mice, although the levels of absorption decrease in complex anthocyanins. Gastric acid does not damage the structure of anthocyanins, allowing them to retain their structure upon reaching the stomach. Absorption begins in the stomach, with 19-37% of anthocyanins being absorbed via the bile translocase mechanism. As a result, anthocyanins can be found in the bloodstream within a few minutes after consumption. Most absorption occurs in the jejunum, with no absorption in the ileum and large intestine. Maximum plasma concentrations are reached within 0.5-2 hours after consumption of anthocyanin-rich fruits. The systemic bioavailability of anthocyanins is estimated at 0.26-1.8% according to the results of animal studies. Meanwhile, maximum plasma levels of total anthocyanins range from 1-100 nmol/L after consumption of berries or grapes in humans.[36]

Anthocyanins are detected in blood as aglycones, glycosides, glucuronides, sulfates, and methyl conjugates. The enterohepatic circulation of anthocyanins can extend their residence time in the body by more than 20 times. More than 200 anthocyanin metabolites have recently been identified in urine, with high levels of these metabolites persisting even five days after consumption.[37] This prolonged presence can compensate for the rapid clearance of anthocyanins from the circulation.[36] Anthocyanins are excreted in the urine in their conjugated forms. Studies on blueberry juice intake have shown that 4% of the anthocyanins excreted in urine are in their parent form, while 96% are in metabolized forms after 24 hours of consumption. Additionally, intact anthocyanins can be found in feces.[37]

Anthocyanins competitively attach to estrogen receptors with endogenous estrogen. If administered alongside estrogen, anthocyanins exhibit antiestrogenic properties by competing with estrogen to attach to estrogen receptors. In contrast, in conditions of low estrogen levels, anthocyanins act as estrogen agonists. In cells with estrogen receptors, anthocyanins can induce the expression of estrogen-dependent gene reporters, leading to cell proliferation. The proliferative effect can be blocked by estrogen receptor antagonists, indicating that anthocyanins possess estrogenic activity.[38]

Anthocyanins in blackcurrants are flavonoids whose have similar structure to flavanones and isoflavones, had been proven to demonstrate estrogenic activity in vitro and in vivo. In vitro studies using fibroblast cell types that were given blackcurrants extract revealed significant increases in type I and type III collagen, elastin, TIMP and decreases in MMP-12 as shown by RT-qPCR examination. Immunohistochemical examination also showed increased levels of collagen I, collagen III, and elastin. In vivo studies with female Sprague–Dawley strain rats after ovariectomy and treatment with 3% blackcurrant extract for three months showed significant improvements in collagen thickness compared to controls. Anthocyanins from blackcurrants have a higher binding affinity to ERβ compared to ERα. In silico docking analysis of cyanidin and delphinidin indicates that the core structure of these compounds corresponds to the ligand binding pockets of ERβ and ERα, suggesting structural similarity to 17β-estradiol or estrogen. In silico docking

analysis also shows that the selectivity of ERβ is higher than that of ERα. Therefore, it can be concluded that anthocyanins have estrogenic activity through their attachment to ERα and ERβ.[2] Clinical studies of the effects of creams, gels and lotions containing the phytoestrogens isoflavones or genistein conducted over 12-24 weeks have shown improvements in skin dryness, thickness, facial wrinkles, fibroblast viability, and increases hyaluronic acid levels and type I and III collagen.[10] In this study, no significant side effects were detected after topical use of formulations using phytochemical active ingredients. Anthocyanins sourced from blackcurrant were able to increase ERα expression and provide estrogen-like effects, inducing the proliferation of columnar epithelial cells in the uterus of immature mice[8]. They also increased the expression of ECM proteins, such as collagen (type I and III) and elastin, in the skin of ovariectomized mice, suggesting that anthocyanins can mitigate skin aging caused by estrogen deficiency or EDS. [4]

4. Conclusion

Skin aging is caused by intrinsic and extrinsic factors. One of the intrinsic factors is a decrease in estrogen hormone levels, a condition known as estrogen-deficient skin (EDS) in menopausal women. This condition results in the formation of wrinkles due to decreased production of collagen and elastin in the skin. While hormone replacement therapy can be used to treat EDS, it is associated with an increased risk of malignancy. As an alternative, natural sources containing anthocyanins, which act as phytoestrogens and SERM, can be used to mitigate skin aging caused by EDS

5. Conflist of Interest

No conflict of interest

6. Author Contribution

All authors contributed to the process of preparing this scientific article

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