Current insight flavonoid quercetin in epithelial maturation vagina for menopause treatment: a narrative review

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ABSTRACT

**Background:** Menopause affects all parts of the women's body system, especially in the genital urinary area. The impact on the genital urinary system causes post-menopausal women to complain of menopause-related genital urinary syndrome due to vaginal atrophy, sexual dysfunction, and pelvic organ prolapse in the long term. Various research has been conducted to find alternative therapy, especially flavonoid quercetin, due to its phytoestrogen characteristic.

**Methods:** This review article assembles much literature to highlight the menopause effect on the vagina and the phytoestrogen effect of flavonoid quercetin as a potential alternative therapy. This study aims to develop future experimental studies of quercetin's effect on the vagina.

**Results:** Hypoestrogenic condition affects all vaginal layers. This is due to estrogen receptors distributed in the epithelial lining, lamina propria, and muscular layer of the vagina. In vitro studies showed quercetin induces cell proliferation by activating signal pathway b-catenin/c-Myc/cyclin A2, mediated by estrogen receptor. The signal pathway increases the cell cycle transition to the G1 phase. It also increases keratinocyte proliferation and migration, proliferating cell nuclear antigen, and positive a6 and b1 integrin cells in the basal layer of the epidermis, which causes thickening of the epidermis.

**Conclusion:** Flavonoid quercetin is a promising alternative for menopause therapy in the vagina. Further, in vivo studies are needed to confirm the result of in vitro study and as basic data for comparison with other potent phytoestrogen.

**Keywords:** Flavonoid quercetin, epithelial, vagina, menopause.

INTRODUCTION

Population aging is a global phenomenon that cannot be ignored. This is due to increased human life expectancy. Between 2000 and 2019, global life expectancy increased from 66.8 to 73.4 years.¹ Life expectancy has increased to 79.3 years in America, while in Indonesia, it has increased from 69.81 in 2010 to 71.57 in 2021.²,³ Currently, Indonesia has an elderly population of 28 million, or 10.8%, which is estimated to increase to 12.5% in 2025.⁴ With the increasing age of women, hormonal conditions, namely menopause, very much determine their quality of life.

Menopause is the end of the female reproductive period, which signifies the permanent cessation of ovarian function. At menopause, there is a 95% decrease in estrogen production, which causes a hypoestrogen condition.⁵ This hypoestrogen condition affects the entire structure and function of body tissues, causing complaints, one of which is in the genital urinary area. Menopause causes structural changes in all vaginal linings, namely epithelial thinning and decreased connective tissue density, smooth muscle, and blood vessels. These changes cause as many as 50% of postmenopausal women to complain of a collection of symptoms known as menopause-related genitourinary syndrome. Some symptoms include reduced vaginal lubrication, itching, easy irritation, decreased libido, dyspareunia, vaginal discharge, recurrent urinary tract infections, urinary incontinence, and dysuria.⁶,⁷ There is also shortening, narrowing, and loss of vaginal elasticity, which causes 93% of postmenopausal women to experience sexual dysfunction and 40% to experience pelvic organ prolapse.⁸,⁹ These changes in the urogenital tract cause discomfort, psychosocial pressure, decreased self-confidence, and a decrease in the overall quality of life for women.¹⁰

Menopausal first-line therapy in the genital urinary system is only symptomatic. It can only treat mild-moderate symptoms, while hormonal therapy requires a long treatment and has side effects such as endometrial hyperplasia, breast cancer, colorectal cancer, and ovarian cancer.⁵ Various alternative therapies were developed to overcome the effects of menopause on the vagina, one of which is flavonoids. Flavonoids are phenolic substances found in various fruit and vegetable plants. Flavonoids are known to have properties as antioxidants, anti-inflammatory, and modulators of cellular enzyme functions. One of the most researched subclasses is Quercetin. Quercetin is commonly found in onions, tea, apples, and berries, is often consumed,
and has high phytoestrogen properties. So, it is starting to be studied further as an alternative menopause therapy option.11,12
Phytoestrogens are non-steroidal compounds of plant origin structurally and functionally similar to the potent mammalian estrogen, namely estradiol. The phytoestrogen quercetin binds to the estrogen receptor (ER), modulates estrogen-regulated gene expression, and exhibits estrogen agonist activity.13,14
The phytoestrogen quercetin stimulates cell proliferation via estrogen receptors, accelerating skin wound healing. Several studies on the skin have shown that quercetin stimulates the proliferation of epidermal stem cells located in the basal layer of the epidermis, increasing the proliferation and migration of keratinocytes.14,17 Cell proliferation by quercetin occurs by stimulating G1/S cell cycle phase changes, which increase cell growth and DNA replication. Quercetin also accelerates wound healing by reducing the inflammatory response and oxidative stress, stimulating re-epithelialization, and suppressing fibrosis formation.18,19
The nature of phytoestrogens that affect re-epithelialization and suppression of fibrosis formation by quercetin is the basis for research as a therapy for vaginal atrophy, prevention, and pelvic organ prolapse.

Much research discussing the phytoestrogenic abilities of quercetin is still in the in vitro research phase. Until now, there has been no discussion of the effect of quercetin on the vagina in vivo. Based on those mentioned above, this study aims to discuss the potential of the flavonoid quercetin in vaginal epithelial maturation as an alternative therapy for menopause.

Pathophysiology of Menopause
The number of oocytes decreases throughout a woman’s life.20 At birth, oocytes number 2 million and decrease to 400,000 primordial follicles at puberty.21 At the age of 37–38 years, women experience a decrease in the number of follicles up to 25,000.22 The decrease in the number of follicles causes the production of inhibin B by granulosa cells to decrease. FSH levels then increase because the negative feedback from inhibin B is reduced. In perimenopause, FSH levels are consistently elevated in the early follicular phase. In the final reproductive phase, high FSH levels accelerate the process of recruitment and follicular development, characterized by increased estrogen levels, which are then followed by a decrease. The decline in inhibin production occurs at the age of 35 and decreases drastically at the age of 40, in line with decreased fertility.23
The menopausal transition period is divided into three phases: the follicular activity phase, which is still normal; the luteal phase insufficiency; and menopause with low levels of estrogen and progesterone.24 Menstrual cycles are getting longer and longer due to delayed ovulation cycles or anovulation. FSH increases 2 years before menopause until it finally plateaus 1 year after menopause. Estradiol decreased drastically two years before menopause.25 Menopause occurs when the number of follicles falls below a critical value of around 1000. By the time menopause has occurred, FSH levels are higher than 60 IU/L, accompanied by increased LH levels of 10–45 IU/L. Other hormone levels decreased, including estrogen <200 pmol/L, progesterone, and inhibin B <25 ng/L.26,27
The ovaries no longer produce estrogen after menopause. Estrogen levels decrease but are maintained by converting androstenedione and testosterone to estrogens. During menopause, androstenedione is mainly produced by the adrenal glands, while testosterone is primarily derived from the peripheral conversion of androstenedione.28–30 These two hormones are also produced by the ovaries during menopause, although in small amounts. Body weight has a positive correlation with the conversion of androstenedione to estrogen.31
The hypoestrogenic condition affects the entire lining of the vagina (Figure 1). In the epithelial layer, epithelial thinning occurs, characterized by a decrease in epithelial thickness and is dominated by immature cells. Clinically, the vagina looks smooth, pale, shiny, and fragile. This condition is known as vaginal atrophy, leaving the vaginal wall vulnerable to trauma. Vaginal irritation, bleeding, and ulceration often occur during sexual activity.32,33

Figure 1. Changes in epithelial structure in menopausal patients.34

The vaginal epithelium produces glycogen. Glycogen is present in the cytoplasm of epithelial cells and increases in mature epithelial cells. In the vagina, there is colonization of the microbiota, which is dominated by the genus Lactobacillus, which converts glycogen into lactic acid. This lactic acid controls vaginal pH, ranging from 3.5 to 4.5, to prevent bacterial vaginosis and urinary tract infection.35,36
During menopause, glycogen production decreases, and the number of Lactobacillus decreases, which results in vaginal pH elevation.37 The risk of infection is 5% at the start of menopause, with an increasing trend of up to 50% at the age of over 80 years.38 Additionally, reduced vaginal lubrication causes itching, burning, and dyspareunia. This is the most frequent and disturbing complaint, especially among sexually active women.39
The lamina propria lining of the vagina also thins during menopause. The extracellular matrix is thinning, consisting of a network of collagen and elastin, which contributes to vaginal biomechanics. Fibroblasts regulate collagen, elastin, and extracellular matrix formation and metabolism.40 In several studies, vaginal fibroblast proliferation has decreased in menopausal conditions.41,42 Hypoestrogenic conditions cause a decrease in the number and quality of
fibroblasts. Fibroblasts appear small, irregular, and long, and the intercellular distance of fibroblasts widens. In terms of quality, there is a decrease in the activation of fibroblasts to become myofibroblasts. This causes a decrease in the ratio of type 1 and type 3 collagen, which has a clinical effect on decreasing tissue elasticity. Clinical manifestations include decreased skin turgor, shrinking vaginal introitus, and shortening and shrinking of the vagina until Pelvic Organ Prolapse (POP) occurs. The incidence of pelvic organ prolapse during menopause is 40% and increases with age.

**Flavonoids**

Flavonoids are hydroxylated phenolic substances with a benzo-pyrene structure. Flavonoids have a chemical structure of a flavan core (C6–C3–C6) and consist of two benzene rings (A and B) connected via three carbon atoms, which form a heterocyclic pyran (C) ring.

Plants form flavonoids as a protective response against microbial infections. Most flavonoids are found in fruit, vegetables, tea, and cocoa plants. In the last two to three decades, flavonoid research has increased rapidly. This is because the flavonoids contained in plants have antioxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic properties. Flavonoids have several subclasses, including flavonols, anthocyanidins, flavanones, flavones, flavanols, isoflavones, and chalcones.

In recent years, research on flavonoids has been widely reviewed, especially those with a role in medical treatment. This is because the flavonoid compounds contained in plants are easy to extract and have a structure that can be easily replaced with glycosides, methyl groups, hydroxyl groups, and sulfates, so they are easily suitable for use in medical treatment. The ring structure of flavonoids has hydrophobic and polar components to interact with amino acid residues, which are the ligand-binding domains of the estrogen receptor.

Flavonoids also can modulate cellular enzyme function. The structural similarity of flavonoids with estradiol makes flavonoids also phytoestrogens. Phytoestrogens have the same activity as estrogen; they bind to estrogen receptors in the body, act as estrogen agonists, change synthesis patterns, metabolize endogenous hormones, and modify the number of hormone receptors, even though their affinity is lower when compared to estradiol.

**Quercetin**

Quercetin (C15H10O7) belongs to the flavonol subclass of flavonoids. These compounds are found in many vegetables, including onions, apples, broccoli, asparagus, kale, red wine, cherries, and tea. Daily consumption of foods with a high quercetin content increases its bioavailability in the body. Several studies have shown that quercetin content in vegetables is not reduced by food processing and that the concentration in blood plasma is positively correlated with the average amount of food consumed.

Quercetin has been widely studied in the last decade due to its high antioxidant, anti-inflammatory, and anti-carcinogenic activities. Other properties of quercetin include anti-carcinogenic, anti-inflammatory, antimicrobial, antiviral, antithrombotic, anti-aggregation, and vasodilation. Due to the many effects of quercetin's bioactivity, it is widely used as a food supplement.

The effect of the phytoestrogen flavonoid quercetin is currently being widely studied. The mechanism of action of phytoestrogens is by binding to estrogen receptors (ER), modulating the expression of estrogen-regulated genes, changing the pattern of estrogen synthesis, and modifying the number of hormone receptors. Many studies have shown that quercetin is a phytoestrogen with a mechanism of action by binding to the ER as an ER ligand, which influences phosphorylation activity in the signal transduction cascade by kinases. Several other studies state how phytoestrogens work apart from binding to ER through cross-talk with different signaling pathways. These signaling pathways include the Epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGF1R), aryl hydrocarbon receptor (AhR), peroxisome...
Quercetin has almost the same affinity for ERα and ER but a lower affinity for estrogen. Research by Van der Woude et al. (2005) showed quercetin's affinity for ERα and ER $10^7$ and $10^6$ lower than estrogen. Nevertheless, quercetin can activate ERα and ER, respectively, 4.5 and 1.7 times greater than estrogen. Although it exhibits a lower affinity for the receptor than estrogen, quercetin in vitro still exhibits phytoestrogen activity due to its receptor affinity. The effect of phytoestrogens on quercetin has a biphasic effect. Phytoestrogens will show estrogenic activity at low concentrations, but at high concentrations, they will show anti-estrogen activity. At low doses, quercetin promotes cell proliferation via the estrogen receptor. Quercetin is a ligand that binds to ERα and β and works as a full-agonist estrogen. After binding to the receptor, quercetin activates transcription and induces cell proliferation. On the contrary, high-dose quercetin causes inhibition of cell proliferation without estrogen receptor stimulation. In research, in vitro doses greater than 10 μM are cytotoxic, with the most visible results at doses of 100 μM. Exposure to high doses in vitro for 24 hours can still be tolerated but has caused maximum transcription stimulation, while on exposure for 3 days, cell death can occur. Multiple mechanisms of the antiproliferative effect of quercetin, such as DNA strand damage, cell cycle arrest, and/or induction of apoptosis through enzyme inhibition associated with signal transduction, namely phosphatidylinositol-kinase, protein kinase C, and protein tyrosine kinase. Quercetin is a competitive inhibitor binding site for ATP phosphatidylinositol kinases, protein kinases, and tyrosine kinases, which play important roles in DNA stimulation, synthesis, and cell mitosis. The effect of cell proliferation inhibition was seen in positive and negative cell tests for estrogen receptors. This biphasic nature is not only possessed by quercetin but also by other phytoestrogen compounds.

**Figure 3.** The pathway of action of Quercetin in stimulating the proliferation of epidermal cells.

Pro?liferator-activated receptors (PPARs), and estrogen-associated alpha/gamma receptors (ERRα/γ) (Figure 2). Compounds that include phytoestrogens besides quercetin contain isoflavones, genistein, isocoumarins, chalcones, coumestans, lignans, and phenolics. Until now, the phytoestrogen content studied in depth has been isoflavones and lignans. This is due to isoflavonoids in many grains, especially soybeans, which are most often consumed in food, and lignans, which are present in almost all plants.

Several studies have been conducted on the isoflavone subclass aimed at general menopausal symptoms as well as in the vagina. A systematic review study of isoflavones from red clover extract shows that it can significantly reduce hot flash symptoms, but vaginal studies only found one study with the results of complaints of vaginal dryness improving by 41%. In a systematic review study of isoflavones in soybeans, conclusions cannot be drawn despite quantitative analysis showing effective results in improving complaints of vaginal atrophy due to publication bias and broad heterogeneity. Another study using the isoflavonoid genistein showed increased vaginal epithelial thickness, although it could not reach vaginal epithelial thickness under normal estrogenic conditions. Lima et al. (2014) conducted a study in humans using isoflavone gel containing 10% dried soybean extract per 1 gram, showing an increase in epithelial thickness, epithelial maturation, and improvement in complaints related to vaginal atrophy after 4 weeks of therapy.

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Most of the phytoestrogens have estrogen antagonist or agonist properties. Several studies regarding the properties of the phytoestrogen quercetin are still being carried out in vitro. Research by Van de Woude et al. (2005) stated that quercetin stimulates cell proliferation through the ER. In vitro studies on the skin showed that quercetin accelerated skin wound healing by activating the ER-mediated β-catenin/c-Myc signaling pathway, increasing cyclins A2 and A1, and stimulating the proliferation of epidermal stem cells in the basal layer of the epidermis (Figure 3).

Other studies also support these findings, showing that quercetin increases keratinocyte proliferation and migration through increased catenin expression. Increased Cyclin A1 and A2 expression by quercetin stimulates changes in the G1/S cell cycle phase, in which cell growth and DNA replication occur. Besides that, it increases proliferating cell nuclear antigen (PCNA) and positive a6 and b1 integrin cells in the basal layer of the epidermis, which causes thickening of the epidermis. Quercetin also accelerates wound healing by reducing the inflammatory response and oxidative stress, stimulating re-epithelialization, and suppressing fibrosis formation.

The limitation of this study is the lack of in vivo research on quercetin in skin wound healing to confirm the ability to stimulate cell proliferation. In vitro research findings indicate a role for quercetin as an alternative therapy. However, further study is needed to compare the effectiveness with other potent phytoestrogens, optimal dose, and side effects.

CONCLUSION

Menopause is a condition where ovarian function stops permanently, which decreases estrogen production by 95%. Hypoestrogen conditions affect the genital organs, especially the vagina, resulting in changes in vaginal structure, including thinning of the epithelium, decreased density of connective tissue, blood vessels, and vaginal smooth muscle which causes vaginal atrophy, shortening and narrowing of the vagina, loss of vaginal elasticity, and prolapse of the genital organs. Until now, the main treatment option is non-hormonal, which is symptomatic, which has the disadvantage of only being able to reduce complaints of mild-moderate symptoms and cannot improve vaginal atrophy and its accompanying complications. On the other hand, hormonal therapy requires a long treatment time and has side effects of endometrial hyperplasia, breast cancer, colorectal cancer, ovarian cancer, and cardiovascular disease. This review examines the flavonoid quercetin as an alternative therapy for menopause. The flavonoid quercetin is a phytoestrogen that binds to estrogen receptor alpha, modulates the expression of estrogen-regulated genes and exhibits estrogen agonist activity. The flavonoid quercetin stimulates cell proliferation, affecting the re-epithelialization of vaginal epidermal cells.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

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REFERENCES


