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Original Article

Expresion of Basic Fibroblast Growth Factor (bFGF) and Cell Apoptosis in Human Pterygium Fibroblast (HPF) after the Adminstration of Epigallocathecin-3-Gallate (EGCG) and Mitomycin-C (*In Vitro* Laboratory Experimental Study)

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ABSTRACT

Pterygium is an ocular disease characterized by the growth of fibrovascular tissue and a wing-shaped fold of conjunctiva on the surface of the cornea. The progression of this disease is primarily influenced by exposure to the ultraviolet (UV) light, which induces the production of growth factors, one of which is basic Fibroblast Growth Factor (bFGF), at the chronic inflammation or DNA damage stages, causing pterygium cell invasion. Various surgical techniques serve as the main modalities for this disease, including conjunctival flaps, conjunctival autografts, bare sclera, and the use of amniotic membranes. No specific procedure can completely prevent the recurrence of this disease. The recurrence of this disease at the global level and in Indonesia remains high, ranging from 10-80% and 24-89%, consecutively. A combination of pterygium treatment with adjuvant therapy developed from natural ingredients was developed to resolve this problem. One of the natural ingredients is epigallocatechin-3-gallate (EGCG), which is the extract of green tea. EGCG is acknowledged to have anti-inflammatory, antioxidant, antifibrotic, and antineoplastic effects. EGCG can moderately attenuate pterygium cell proliferation without significantly affecting conjunctival cells. Therapy using EGCG can be applied in various techniques that effectively meet the EGCG needs in the body and treat eye diseases. So far, there have not been many studies on the effect of EGCG administration on pterygium recurrence. For those reasons, it is necessary to conduct a study regarding the effect of the administration of EGCG on the expression of bFGF and apoptosis in Human Pterygium Fibroblast (HPF) compared to the administration of mitomycin C (MMC), which is the commonly used therapy. It is expected that EGCG administration can serve as the best solution for preventing pterygium recurrence in the future.

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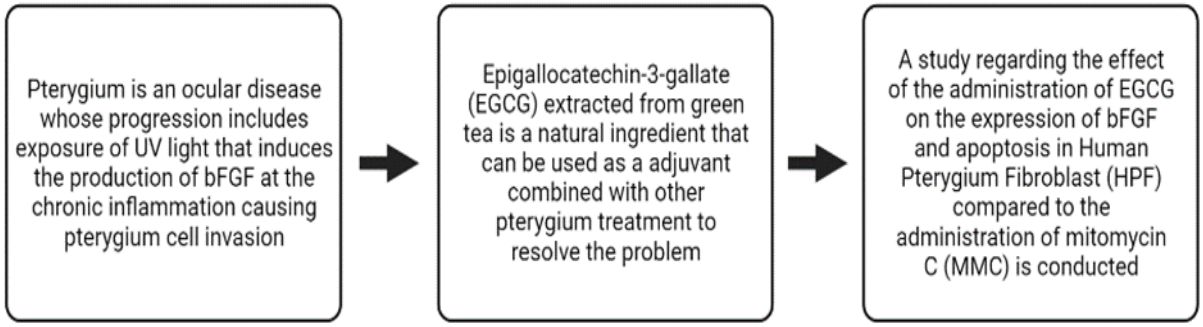
GRAPHICAL ABSTRACT



Introduction

Pterygium is a common ocular disease [1-3]. The global prevalence of the disease ranges from 1.1% to 53%. Meanwhile, according to the 2010 Basic Health Research data, the prevalence of this disease in Indonesia reached 3.2% affecting both eyes, and 1.9% affecting one eye. Besides, the recurrence rate of this disease is relatively high, ranging from 10-80% globally and 24-89% in Indonesia [4]. The high recurrence rate and progression rate of recurring pterygium remains a challenging clinical problem for ophthalmologists in Indonesia and has not been resolved [5-7]. Therefore, new treatment strategies are needed to address the problem.

A combination of pterygium treatment with adjuvant therapy developed from natural ingredients, with minimal side effects but greater benefits, can be considered in treating eye diseases [8]. One of the natural ingredients is epigallocatechin-3-gallate (EGCG), the extract of green tea. Studies have shown that EGCG can effectively reduce the recurrence and migration rates of pterygium fibroblast cells in laboratory settings, without causing harm to conjunctival cells [9]. As a result, the EGCG administration is proposed as a promising and innovative therapy for primary pterygium treatment [10]. Furthermore, postoperative adjuvant therapy with EGCG holds potential as a successful approach for managing pterygium recurrence. In the future, EGCG could be formulated into eye drops, offering an alternative therapeutic option for this condition.

Results and Discussion

Pterygium

Pterygium is a degenerative condition affecting the wing-shaped conjunctiva [8]. It typically manifests as a triangular growth of fleshy fibrovascular tissue extending from the bulbar conjunctiva onto the cornea, primarily on the nasal side. The primary cause of pterygium is exposure to ultraviolet (UV) radiation (Figure 1) [8]. This condition is among the most prevalent corneal disorders, leading to a decline in visual acuity. To assess pterygium, certain criteria should be considered, including its location, size, vascularity, extent, and the area of corneal involvement (measured using Castroviejo's calipers to determine the chord at the limbus and the degree of corneal encroachment from the limbus). A pterygium consists of three components: the cap, which is found at the leading edge and characterized by an avascular zone resembling a halo; the head, located peripherally to the cap; and the body, the primary portion of the pterygium that connects with the bulbar conjunctiva. Pterygium can be classified as progressive, exhibiting thickness, fleshy appearance, high vascularity, and progressive growth towards the center of the cornea; or atrophic, which is characterized by thinness, attenuation, poor vascularity, and a stationary state [2].

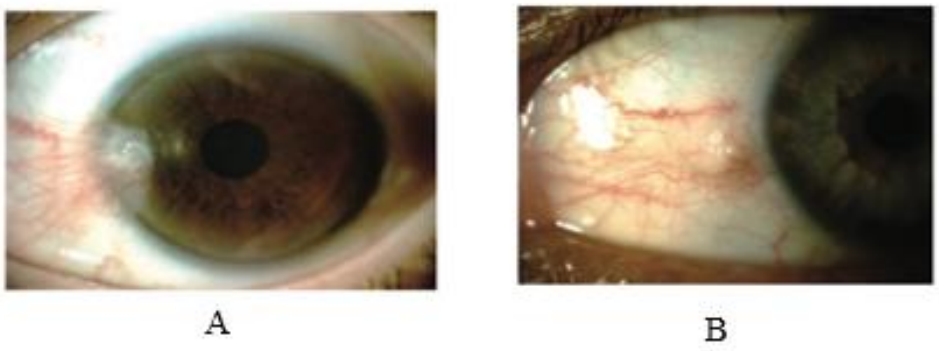


Figure 1: Pterygium. (A) Nasal pterygium and (B) Nasal and temporal (double-headed) pterygium [2]

The recurrence of pterygium is influenced by various factors, including but not limited to the UV radiation exposure, environmental irritants (such as dust and wind), viral agents, genetic factors, as well as immunological and inflammatory factors. Recent investigations have also highlighted additional risk factors, such as the presence of specific gene transcription factors like cAMP response element binding protein (CREB), phospholipase D, cytochrome P450 (CYP) 1A1, aquaporin-1, and aquaporin-3. The pterygium pathogenesis involves a complex interplay of these underlying factors. Nonetheless, it is crucial to emphasize that sunlight exposure remains the predominant risk factor, significantly contributing to the initiation and progression of pterygium [2].

Pterygium development is influenced by hereditary factors and sun exposure (Figure 2). Hereditary factors impact the growth factors involved in fibroblast division. Inflammation-induced fibroblast mitosis generates a large number of fibroblasts that collectively produce collagen [11]. The excess collagen is invaded by fibroblasts, and the stimulation of growth factors and ROS leads to the formation of new blood vessels, contributing to pterygium formation.

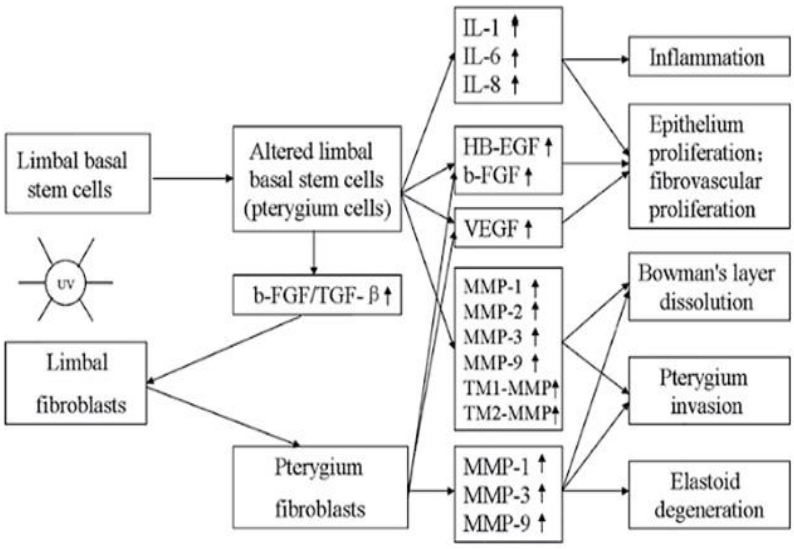


Figure 2: The potential role of the UV light in the pterygium pathogenesis. Exposure to the UV light can induce changes in limbal stem cells, initiating the formation of pterygium. Pterygial cells, characterized by increased expression of inflammatory cytokines, growth factors, and MMPs, contribute to inflammation, fibrogenesis, vascularization, and pterygium invasion. The UV radiation or interactions between pterygium cells and limbal fibroblasts in a bFGF/TGFβ-dependent manner activate the fibroblasts, leading to elevated levels of various growth factors and MMPs. This process results in remodelling of the extracellular matrix, dissolution of Bowman's membrane, and pterygium invasion

Collagenolysis facilitates the invasion of new fibroblasts and blood vessels into the stroma. The pterygium occurrence at the surgical limbus is attributed to the abundance of endothelial cells, which generate ROS upon sun exposure [4].

One widely accepted hypothesis regarding pterygium pathogenesis is that exposure to the environmental factors, particularly the UV radiation, can activate various signaling pathways that trigger the production of mediators responsible for pterygium growth [1]. At the molecular level, the UV radiation is associated with the generation of active free radicals that can damage and deactivate macromolecules. DNA methylation can also regulate matrix remodeling and cell adhesion, influencing the formation of epithelial pterygium. Furthermore, pterygium may exhibit resistance to the UV radiation-induced apoptosis, which can be attributed to an imbalance between proapoptotic and antiapoptotic proteins [12].

Basic fibroblast growth factor (bFGF)

Cell proliferation is a prominent characteristic in the development of pterygium. Numerous growth factors have been investigated, and a comprehensive list of these molecules, along with a brief overview of their roles as pathogenic factors, is presented in Table 1. Among the growth factors affected by the UV-B radiation is bFGF, also known as fibroblast growth factor 2 (FGF2) [13]. This factor plays a significant role in angiogenesis, wound healing, and various endocrine signaling pathways [14, 15]. Studies have demonstrated increased expression of bFGF in infiltrating mast cells, epithelial cells, and blood vessels associated with pterygium. Mast cells can also act as an additional source of bFGF. Detorakis *et al.* conducted qRT-PCR analysis to measure FGF2 mRNA levels in normal pterygium and conjunctiva, revealing higher expression of FGF2 in pterygium compared to normal tissue [16]. This upregulation of FGF2 can induce the expression of cyclooxygenase-2 (COX-2), an enzyme absent in normal conjunctiva, but present in human pterygium fibroblasts. COX-2 expression is crucial for inflammatory cytokine-induced angiogenesis, suggesting that FGF2 contributes to inflammation and angiogenesis in pterygium [16, 17].

Pterygium fibroblast cell apoptosis-associated protein

Survivin, Bcl-2, Bax, and Bcl-w are examples of proteins implicated in the regulation of cell apoptosis. Survivin, a significant member of the apoptosis inhibitor family, plays a role in inhibiting caspase activation, thereby negatively regulating apoptosis. Research by Liu *et al.* demonstrated strong expression of survivin in pterygium tissue, with localization in both the nucleus and cytoplasm of epithelial cells. In contrast, normal conjunctival epithelium showed weak expression limited to the cytosol. Knockdown of survivin was found to suppress the propagation of pterygium epithelial cells and correlated with the downregulation of p63 expression, as well as the upregulation of p57 and p21 expression. This suggests that oxidative stress can trigger the activation of survivin expression, leading to a hyperproliferative state that may contribute to pterygium growth [17].

Table 1: Growth factor and its role in the pathogenesis of pterygium [18]

	Abnormalities	Pathogenic potential
bFGF	Increases after UVB radiation	Triggers cell proliferation
PDGF	Overexpression	Triggers cell proliferation
TGF-β	Overexpression	Triggers cell proliferation and inflammation
HB-EGF	Increases after UVB radiation	Trigger cell migration
PEDF	Declines	Triggers angiogenesis
VEGF	Increases after UVB radiation	Triggers angiogenesis
8IGFBP-2	Overexpression	Triggers proliferation
Stem cell factor	Overexpression	Triggers angiogenesis
CTGF	Overexpression	Triggers changes in connective tissue

Adjuvant therapy

The high recurrence rate associated with surgery remains a problem. Thus, adjuvant medical therapies have been incorporated into the postoperative management of pterygium. Many sources have indicated that the addition of these therapies leads to a dramatic decline in the relapse rate of the disease. It is expected to serve as a highly-effective and reliable alternative treatment in the future.

Mitomycin C (MMC)

Mitomycin C (MMC) is an antimicrobial/anticancer agent derived from Streptomyces caespitosus. Since 1963, it has been proposed as an adjunctive treatment for pterygium surgery. The combination of conjunctival autograft with MMC, irrespective of the dosage or method of application, has demonstrated substantial efficacy in reducing pterygium recurrence. Furthermore, postoperative administration of MMC through topical eye drops is a viable approach. However, it is crucial to consider the potential complications associated with MMC usage, including the development of chronic keratopathy and toxic keratoconjunctivitis. To ensure better control of the dosage, intraoperative administration of MMC is recommended [19].

Some studies have investigated the effectiveness of utilizing preoperative subconjunctival injection of MMC as an adjunct therapy for pterygium surgery. In a prospective study involving 36 patients, a subconjunctival injection of 0.1 ml-0.15 mg/ml MMC was administered into the pterygium head one month prior to surgical excision. The study followed up for a duration of two years, during which a recurrence rate of 6% was observed, and no complications related to wound healing were documented. Another randomized controlled trial (RCT) study involved 50 eyes with recurrent pterygium, which were divided into two groups: a preoperative MMC injection group and a postoperative topical MMC group. In the preoperative group, a 0.1 ml-0.15 mg/ml MMC injection was administered the day before the pterygium excision surgery. The difference in relapse and complication rates between the two groups did not yield statistically significant results. As a result, the authors concluded that preoperative low-dose subconjunctival injection of MMC is an effective approach for managing recurrent pterygium [2].

Epigallocathecin-3-Gallate (EGCG)

Green tea derived from Camellia sinensis contains a diverse range of flavonoids, with catechins being a major class of flavonoids, including epigallocatechin gallate (EGCG) (Figure 3). EGCG is the most abundant catechin and has demonstrated anti-inflammatory and antioxidant properties in various cell types. Recent studies conducted *in vivo* and *in vitro* have also highlighted the antivasodilating, anticarcinogenic, and neuroprotective effects of EGCG [9].

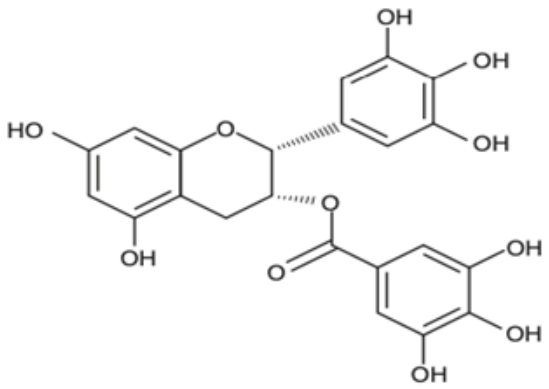


Figure 3: Epigallocathecin-3-gallate (EGCG) chemical structure [6]

Pterygium is a condition affecting the eyes and characterized by the development of triangular hyperplastic growth, conjunctivalization, inflammation, and remodeling of connective tissue. The cellular properties of pterygium cells bear a resemblance to those of neoplastic cells. Within green tea, there exists a catechin known as EGCG, which exhibits antitumorigenic properties. The effects of green tea catechins on the survival and migration of human primary pterygium cells differ from their impact on conjunctival cells. Both human primary pterygium cells and conjunctival cells express the EGCG receptor, specifically the 67 kDa laminin receptor. Multiple studies have provided evidence demonstrating the significant inhibitory effect of green tea extract and EGCG on pterygium cell proliferation while having minimal impact on conjunctival cells. These findings hold promise for the development of a potential novel therapeutic approach for the primary treatment of pterygium in the future [10].

Studies have observed that the administration of 50 µg/ml of EGCG can suppress the expression of basic fibroblast growth factor (bFGF), an angiogenic factor, as well as reduce the level of vascular endothelial growth factor (VEGF). These two growth factors play a role in the formation of fibrous tissue [20]. Kapoor *et al.* demonstrated that the application of 50 µl of EGCG at a concentration of 0.8 mg/ml using a full-thickness skin incision model in rats significantly improved the quality of wound healing and led to the formation of non-excessive fibrotic tissue [21]. The ddditional roles of EGCG are presented in Table 2.

This study only evaluates the direct pathway of EGCG effects on bFGF expression and fibroblast cell apoptosis in HPF, which could be considered as a limitation. Further research is required to analyze the specific pathways associated with the effect of EGCG treatment on pterygium recurrence. The detailed pharmacodynamic pathway of EGCG needs to be investigated in future developmental studies. However, despite these limitations, this research serves as a pilot study on the EGCG potential for bFGF expression and fibroblast cell apoptosis in HPF, as compared to the standard adjuvant therapy, namely MMC.

Table 2: Mechanism of Epigallocatechin-gallate (EGCG)

Action of EGCG	Pathway and Factors	
	↑	↓
Cell-cycle proteins	P21, pRb	CDK, cyclin D, PgP, and BCRP
Pro- and antiapoptosis	Bax and Bak, Cas3 and Cas9, PARP, Cas8, and Trail	Bcl2, Bclcl, and ID2
Transcription factors	p53, IκB, Nrf2	AP-1, NF-κB, c-jun & c-fos, STAT-1, STAT-3, STAT-5, Elf1, and Wnt/β-catenin
Metastasis	TIMP, COX2, NO	MMP1, MMP3, MMP4, MMP9, MMP13, IL-8, IL-6, VEGF, EGF, and HGF
Protein kinases	Erk1/2, AMPK, cytochrome c, and cGMP/ PP2A	HER2, JAK2, JNK, PKA, PKC, PI3K/Akt/Foxo1, and Ras/MAPK

Conclusion

Pterygium is an ocular disease characterized by the growth of fibrovascular tissue on the corneal surface, primarily prevalent in tropical and subtropical regions with high sun exposure. The recurrence of pterygium is influenced by multiple factors. Ultraviolet (UV) radiation, particularly UV-B, indirectly contributes to the inflammatory process, oxidative stress, increased angiogenic factors, and production of growth factors (such as bFGF) in pterygium. These factors are associated with chronic inflammation, DNA damage, and processes involving migration, differentiation, cell proliferation, apoptosis, and angiogenesis, leading to the inflammation of pterygium cells. To minimize pterygium recurrence, treatment strategies can incorporate adjuvant therapy developed from natural ingredients with minimal side effects, such as EGCG. EGCG possesses anti-inflammatory, antioxidant, antifibrotic, and

antineoplastic properties, which are relevant concerning the neoplastic characteristics of pterygium. EGCG has the potential to reduce bFGF expression and induce apoptosis in HPF cells, offering a promising avenue for preventing pterygium recurrence.

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No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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References

[1]. Djelantik A.S., Yuliawati P., Naryati L., Prevalence and Risk Factors of Pterygium Occurrence in Population above 50 Years Old in Bali, *Bali Medical Journal*, 2016, **5**:528 [Crossref], [Google Scholar], [Publisher]

[2]. Shahraki T., Arabi A., Feizi S., Pterygium: an update on pathophysiology, clinical features, and management, *Therapeutic Advances in Ophthalmology,* 2021, **13**:251584142110201 [Crossref], [Google Scholar], [Publisher]

[3]. Abdurrauf M., Ramadhan F., Zuhria I., Tambunan B.A., Notobroto H.B., Surakhman B., Komaratih E., Mitomycin C, curcumin, and fibrin glue inhibit the cell proliferation and expression of TGF-β in human pterygium fibroblast, *Bali Medical Journal*, 2022, **11**:228 [Crossref], [Google Scholar], [Publisher]

[4]. Anguria P., Kitinya J., Ntuli S., Carmichael T., The role of heredity in pterygium development, *International journal of ophthalmology,* 2014, **7**:563 [Crossref], [Google Scholar], [Publisher]

[5]. Erry E., Mulyani U.A., Susilowati D., Distribusi Dan Karakteristik Pterigium Di Indonesia, *Buletin Penelitian Sistem Kesehatan*, 2012, **14**:21259 [Crossref], [Google Scholar], [Publisher]

[6]. Jin Y., Xi C., Qin J., Yong J., Molecular pathways, green tea extract,(−)-epigallocatechin gallate, and ocular tissue, In *Handbook of Nutrition, Diet, and the Eye*, Academic Press, 2019, 303-320 [Crossref], [Google Scholar], [Publisher]

[7]. Gulani A.C., Gulani A.A., Cosmetic pterygium surgery: techniques and long-term outcomes, *Clinical Ophthalmology*, 2020, **14**:1681 [Google Scholar], [Publisher]

[8]. Sutyawan E., Niti S., Widiana R., Effect of conjunctiva-limbus transplantation with fibrin glue compare to suture technique on stability of the graft attachment in pterygium surgery, *bali medical journal*, 2013, **2**:27 [Crossref], [Google Scholar], [Publisher]

[9]. Rosita E., Prasetyo S.A., Riwanto I., Atmodjo W.L., The effect of Epigallocatechin-3-Gallate (EGCG) combined with low dose sorafenib in apoptosis and Platelet-Derived Growth Factor Receptor (PDGFR) expression in hepatocellular carcinoma rats, *Bali Medical Journal*, 2022, **11**:216 [Crossref], [Google Scholar], [Publisher]

[10]. Yang Y., Shao-Lang C., Yanxuan X., Yao Y., Jia-Jian L., Li W., Vishal J., Xinghuai S., Di M., Tsz K.N., Green tea catechins attenuate human primary pterygium cell survival ad migration via modulation of ERK p42/p44 and p38 pathways, *Journal of Agricultural and Food Chemistry*, 2021, **69**:12209 [Crossref], [Google Scholar], [Publisher]

[11]. Fileanugraha A., Budipramana V.S., Setiawan A., The comparison of fibroblast count and collagen density score in ileal anastomosis with continuous and interrupted suture technique on New Zealand Rabbit, *Bali Medical Journal*, 2019, **8**:S557 [Crossref], [Google Scholar], [Publisher]

[12]. Liu T., Liu Y., Xie L., He X., Bai J., Progress in the pathogenesis of pterygium, *Current Eye*

Research, 2013, **38**:1191 [Crossref], [Google Scholar], [Publisher]

[13]. Worung I.M., Lestari A.A.W., Kandarini Y., Wande I.N., Wirawati I.A.P., Mahartini N.N., Correlation between serum levels of Fibroblast Growth Factor-23 (FGF-23) and parathyroid hormone levels in predialysis Chronic Kidney Disease (CKD) patients at Sanglah General Hospital, Bali, Indonesia, *Bali Medical Journal*, 2021, **10**:830 [Crossref], [Google Scholar], [Publisher]

[14]. Hidayat A., Arief M., Wijaya A., As'ad S., Angiogenesis in Ischemic Stroke Patients Aged 30 - 80 years at Gatot Subroto Army Central Hospital (RSPAD) Jakarta, *Bali Medical Journal*, 2016, **5**:7 [Crossref], [Google Scholar], [Publisher]

[15]. Kartini D., Panigoro S.S., Alam I.A., Dien A.W., Soemarko D.S., Hellyanti T., Wardhana J.D., Marcevianto K.V., Fibroblast growth factor 2 expression on lymph node metastasis in early-stage breast cancer, *Bali Medical Journal*, 2023, **12**:621 [Crossref], [Publisher]

[16]. Detorakis E.T., Demetrios A.S., Pathogenetic mechanisms and treatment options for ophthalmic pterygium: trends and perspectives (review), *International Journal of Molecular Medicine.* 2019, **23**:439 [Crossref], [Google Scholar], [Publisher]

[17]. Feng Q.Y., Hu Z.X., Song X.L., Pan H.W., Aberrant expression of genes and proteins in pterygium and their implications in the pathogenesis, *International Journal of Ophthalmology,* 2017, **10**:973 [Crossref], [Google Scholar], [Publisher]

[18]. Cardenas-Cantu E., Zavala J., Valenzuela J., Valdez-García J.E., Molecular basis of pterygium development, *Seminars in Ophthalmology,* 2016, **31**:567 [Crossref], [Google Scholar], [Publisher]

[19]. Sakti F.K., Updates on the Mechanism and Management of Pterygium: A Brief Review, *European Journal of Medical and Health Sciences*, 2021, **3**:6 [Crossref], [Google Scholar], [Publisher]

[20]. Nuzzi R., Tridico F., How to minimize pterygium recurrence rates: clinical perspectives, Clin Ophthalmol., 2018, **12**:2347 [Crossref], [Google Scholar], [Publisher]

[21]. Kapoor M., Howard R., Hall I., Appleton I., Effects of epicatechin gallate on wound healing and scar formation in a full thickness incisional wound healing model in rats, *The American journal of pathology*, 2004, **165:**299 [Crossref], [Google Scholar], [Publisher]

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