

Epigallocatechin gallate effect on Interleukin-1 β and MMP-9 expression as *Pseudomonas aeruginosa* keratitis adjuvant therapy



Tarosa Yodia Urolita¹, Fariztah Sukainah Nur Fathimah¹, Annisa Karima¹,
Nurwasis^{*}, Ismi Zuhria¹

ABSTRACT

Background: Keratitis *Pseudomonas aeruginosa* is the most frequently occurring eye infection. It is a rapidly developing condition that can lead to ocular infections (endophthalmitis) and corneal perforation resulting in vision loss. Although prompt administration of antibiotics can effectively kill the bacteria, the corneal damage caused by toxins persists. The following inflammatory process results in corneal thinning, fibrosis, and eventual perforation.

Methods: This study is a literature review to support future experimental studies which will be conducted to examine the expression of IL-1 β and MMP-9 on *Pseudomonas aeruginosa* keratitis after administration of EGCG.

Results: Interleukin-1 β is a proinflammatory cytokine secreted by various immune cells in response to acute and chronic inflammation. At the same time, matrix metalloproteinase (MMP) is a group of proteolytic enzymes involved in the pathophysiology of various ocular surface diseases. Epigallocatechin gallate (EGCG) is the major polyphenol compound in green tea, produced from the *Camellia sinensis* plant. Recent studies suggest that EGCG has anti-inflammatory and antioxidant effects on various cell types, including the cornea. EGCG inhibits IL-1 β , which induces the activation of the NF- κ B signaling pathway, which may explain the decrease in uPA expression and the reduction of collagen degradation by corneal fibroblasts. EGCG inhibits the activation of pro-MMP 9 produced by corneal fibroblasts in response to IL-1 β stimulation, and this effect is mediated by the inhibition of uPA upregulation without a significant impact on pro-MMP 9 expressions.

Conclusion: Epigallocatechin gallate (EGCG) in *Camellia sinensis* extract has the potential as an alternative adjuvant therapy for bacterial keratitis because it has antibacterial, anti-inflammatory and antioxidant effects.

Keywords: Keratitis, Epigallocatechin Gallate, Corneal Ulcer, *Pseudomonas aeruginosa*.

Cite This Article: Urolita, T.Y., Fathimah, F.S.N., Karima, A., Nurwasis., Zuhria, I. 2023. Epigallocatechin gallate effect on Interleukin-1 β and MMP-9 expression as *Pseudomonas aeruginosa* keratitis adjuvant therapy. *Bali Medical Journal* 12(2): 1691-1695. DOI: 10.15562/bmj.v12i2.4382

¹Ophthalmology Department, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia.

^{*}Corresponding author:

Nurwasis;
Ophthalmology Department, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;
nurwasispm@yahoo.com

Received: 2023-03-28

Accepted: 2023-05-02

Published: 2023-05-27

INTRODUCTION

Keratitis is an inflammation of the cornea characterized by corneal edema, infiltration of inflammatory cells, and ciliary congestion. Microbes are the most common cause of keratitis and greatly concern developing countries. *Pseudomonas aeruginosa* causes infection and inflammation of the cornea, progressively damaging the cornea and leading to permanent loss of vision. An increase in keratitis caused by *Pseudomonas aeruginosa* is often found in contact lens users, especially those using extended soft contact lenses.^{1,2} *Pseudomonas aeruginosa* is a gram-negative bacterium commonly referred to as an opportunistic pathogen and is an important pathogen in humans.³ The characteristic corneal lesion in

Pseudomonas aeruginosa keratitis is a corneal ulcer with a ring-shaped abscess. This lesion develops rapidly and often results in corneal perforation leading to blindness. Morphologically, the characteristic lesion is liquefactive necrosis and massive polymorphonuclear leukocytes (PMN) accumulation. Infection of the cornea with *Pseudomonas aeruginosa* induces epithelial edema, inflammation, stromal infiltration/destruction, ulceration, and ultimately vision loss.³ The worldwide prevalence of keratitis in contact lens wearers, as determined by corneal scraping, was positive in 63.3% of cases. Among these cases, 30% were caused by gram-negative bacteria, with *Pseudomonas aeruginosa* being the most common. In developing countries, *Pseudomonas aeruginosa* remains the

most frequent organism causing keratitis in contact lens wearers. Approximately 140 million people worldwide who wear contact lenses are at risk of *Pseudomonas aeruginosa* keratitis, which can cause blindness and visual impairment.³

Interleukin-1 β is a proinflammatory cytokine secreted by various immune cells in response to acute and chronic inflammation. Bacterial keratitis is often associated with risk factors that disrupt the integrity of the corneal epithelium. Common predisposing factors include contact lens wear, trauma, contaminated eye medications, compromised defense mechanisms, and altered corneal surface structure.²

Epigallocatechin gallate (EGCG) is a major polyphenolic compound found in green tea produced from the *Camellia*

sinensis plant. Recent studies have reported that EGCG has anti-inflammatory and antioxidant effects on various types of cells, including the cornea.¹

The effect of EGCG on the cornea, especially in keratitis, has not been widely studied. Based on those mentioned above, this literature review aims to evaluate the effect of EGCG on the cornea through the expression of interleukin-1b and MMP-9 as an indicator of the acute phase in keratitis.

Cornea

The cornea is a transparent and avascular tissue composed of the epithelium layer, Bowman's membrane, stroma, Descemet's membrane, and endothelium. In adults, the cornea has a horizontal size of 11-12 mm and a vertical size of 10-11 mm, with a thickness of 500-600 µm in the central area and becoming thicker towards the periphery. The cornea obtains its nutrition from glucose diffusion from the aqueous humor, oxygen in tears, and oxygen from the limbus, especially for the peripheral cornea.³

The cornea's outermost layer is the corneal epithelium, which consists of 4-6 layers, including 1-2 layers of superficial squamous cells, 2-3 layers of broad wing cells, and an innermost layer of columnar basal cells. The epithelium is approximately 40-50 µm thick, representing 5-10% of the total corneal thickness, and has a high regenerative capacity. Continuous proliferation of the limbal stem cells results in the differentiation of the outermost layer into superficial squamous cells. These cells then undergo maturation, leading to the formation of microvilli on their outer surface, which subsequently sloughs off as tears. The differentiation process takes approximately 7-14 days. The basal epithelial cells secrete a basal membrane with a thickness of 50 nm, composed of type IV collagen, laminin, and other proteins.^{3,4}

The Bowman's layer is located in the anterior stroma. It consists of acellular cells and collagen tissue with a thickness of 15 µm that maintain the cornea's shape. This layer is quite resistant and cannot regenerate if damaged. The stroma is the thickest layer, about 90% of the cornea, which consists

of stromal cells (keratocytes), fibers, and extracellular matrix (composed of type I, type V, and type VI fibrillar collagens as well as proteoglycans such as decorin and lumican). Keratocytes produce glycosaminoglycans (GAGs) and matrix metalloproteinases (MMPs), both of which maintain the thickness and integration of the cornea. The Dua's layer, described by previous studies, is a thin layer beneath the corneal stroma containing cells and collagen tissue similar to the corneal stroma.^{2,3}

The stroma, or substantia propria, comprises 90% of the corneal thickness. The stromal composition consists of 80% water, which gives the cornea its viscoelastic properties. The stroma layer comprises keratocyte cells and an extracellular matrix, which contributes to the cornea's clarity. Keratocytes vary in size and density, and their density decreases by 0.9% in the anterior and 0.3% in the posterior layer every year. The anatomical arrangement of collagen fibers and their homogeneous spacing make the cornea appear transparent. However, if the diameter or spacing of collagen fibers becomes heterogeneous, light entering the cornea will scatter randomly, causing a loss of transparency. One example of this is fibrosis or edema in the cornea.³⁻⁵

The Descemet membrane is the basal membrane of the endothelium with a thickness of 3 µm at birth that increases to 10-12 µm in adulthood. Similar to the Bowman's layer, this is resistant and cannot regenerate if damaged. The corneal endothelial cells are located in the posterior part of the cornea and consist of a mononuclear layer containing tightly integrated hexagonal-shaped cells. These cells cannot replicate if there is a wound and tend to change their size to fill the gap in the wound.^{2,3}

The endothelial layer consists of a single layer of polygonal cells. The endothelium is a barrier for aqueous humor and has a metabolic pump function of Na⁺/K⁺ ATPase in regulating hydration. Endothelial cells have limited proliferation, so if they are lost due to trauma or surgery, the defect area will be aided by the remaining cells in the vicinity or peripheral stem cells.^{3,4}

The wound-healing process of the cornea

The cornea can regenerate cells and tissues in each layer, especially in case of damage. There are four stages in wound healing in the corneal epithelium: the latent phase, migration phase, proliferation phase, and epithelial reattachment. The first stage is the latent phase, which occurs from the first hour after injury and is characterized by cells changing shape. Damaged cells undergo apoptosis and are removed by the tear film. The tissue originally formed by gap and adherence junctions is lost due to the wound, leaving damaged basal cells at the wound's edge. Subsequently, the size and shape of the tissue will change and form filopodia and lamellipodia as the basis for epithelial cells to pass through the wound.^{3,4}

The migration phase is the second stage which lasts for 24 to 36 hours, depending on the size and location of the wound. Re-epithelialization occurs at an average rate of 2 mm per day. During this stage, temporary attachment occurs, and the forming material begins to move from the wound edge to the attachment site until the wound is completely covered (Patel, 2019; Liu & Kao, 2015). If the thin layer of the anterior corneal stroma is lost due to abrasion, the epithelial cells will fill the shallow hole, forming a facet. The Bowman layer does not regenerate when damaged. Corneal stroma healing is avascular and occurs through a fibrotic process rather than the fibrovascular proliferation seen in other tissues.^{3,4}

The third stage is the proliferation phase. A layer of cells begins to reconstruct the thickness of the epithelial layer. Basal cells transform wing cells into flattened cells, thus improving corneal function.⁴⁻⁷ In a central corneal injury, neutrophils arrive at the wound through the tear and the wound edges become swollen. The corneal matrix glycosaminoglycans, such as keratan sulfate and chondroitin sulfate, are destroyed at the wound edge. Stromal keratocytes (fibroblast-like cells) are activated and eventually migrate across the wound, forming collagen and fibronectin.⁷⁻¹⁵ The keratocyte spacing is irregular, and collagen fibers are not aligned with stromal lamellae. Therefore,

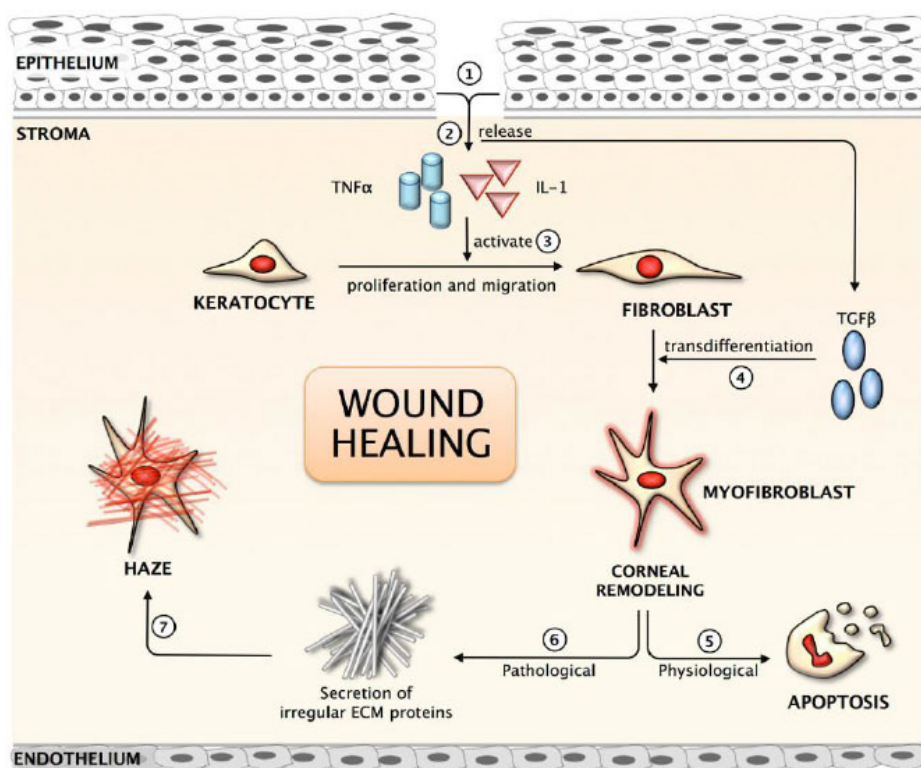


Figure 1. Schematic drawing of the mechanism of corneal wound healing. (1) injury to the cornea resulting in loss of the basement membrane; (2) release of proinflammatory cytokines into the anterior stroma; (3) activation of keratocyte cells to become fibroblasts; (4) TGF- β released by the epithelium then transforms fibroblasts into myofibroblasts; (5) under physiological conditions, myofibroblasts undergo apoptosis followed by corneal repair; (6) in pathological conditions, myofibroblasts secrete irregular matrix; (7) clinically it will show corneal opacities in the anterior stroma.¹⁹

the cells are directed anterior and posterior to cross the wound, which will always appear microscopically as irregularity in the stroma and clinically as an opacity. If the wound edges do not properly close, proliferating keratocytes will not fill the gap, resulting in focal stromal thinning.¹⁵⁻¹⁹

The final phase is characterized by tight cell binding, where hemidesmosomes bind fibrils to attach the epithelial layer to the substrate. The process will occur within a few days if the basal membrane is intact. However, the healing process may take several months if the damage reaches the basal membrane.⁴⁻⁸

Keratitis Pseudomonas

Bacterial infection in the eye is a common condition that threatens vision. In some cases, the onset and progressive stromal inflammation are rapid. Bacterial keratitis is often associated with risk factors that disrupt the integrity of the corneal

epithelium, namely contact lens use, trauma, contaminated eye drops, impaired immune defense mechanisms, and changes in corneal surface structure.⁵⁻⁹

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacterium that commonly contaminates water and is a severe opportunistic pathogen that plays a role in several severe eye infections, such as bacterial keratitis and endophthalmitis. Contact lens-related keratitis is the most frequently encountered *P. aeruginosa* eye infection, particularly in soft and extended-wear contact lens users. Visual impairment is a subsequent consequence of scarring in the cornea and, in severe cases, can cause corneal perforation or extension into surrounding tissues. Some factors that mediate the virulence of *P. aeruginosa* are flagella, adhesins, toxin secretion, proteases, and pili.⁶⁻¹⁰

In general, healthy eyes are protected from *P. aeruginosa* infection. Although

in some studies conducted on corneal epithelial cell cultures, *P. aeruginosa* isolates were found to easily invade and kill them, invasive and cytotoxic *P. aeruginosa* inoculation on intact rat corneas in vivo resulted in rapid bacterial clearance from the ocular surface without pathology. This suggests that specific defense mechanisms against infection in healthy eyes are not found under laboratory culture conditions (in vitro). Previous studies on rabbit models have shown that *P. aeruginosa* is not detected in the cornea within 4 hours of inoculation unless induced by trauma.⁷⁻¹⁵

Contact lenses increase the risk of corneal infection due to several factors, such as prolonged exposure to contaminated foreign bodies, disruption of the tear film, microtrauma to the corneal epithelial surface, impaired immune function on the ocular surface, hypoxia of the cornea due to contact lens use, and improper eye hygiene that leads to inoculation on the ocular surface. The eye is also susceptible to *P. aeruginosa* infection in pathological conditions such as Herpes simplex infection, immunocompromised states, and eye trauma. Following inoculation of the eye with *P. aeruginosa*, attachment to the epithelium occurs, followed by invasion into the corneal stroma and subsequent proliferation, often exacerbated by the host's immune response.⁶

Interleukin-1 β

Interleukin-1 β (IL-1 β) is a proinflammatory cytokine secreted by various immune cells in response to acute and chronic inflammation. Bacterial infection stimulates the recruitment of polymorphonuclear leukocytes (PMNs) to the injury site by activating the innate immune response. PMNs then secrete IL-1, which is key in regulating the host response to bacterial infection, including that associated with corneal ulcers. IL-1 contributes to tissue damage by stimulating the production of the chemokine IL-8 in corneal fibroblasts, which prolongs PMN infiltration into the cornea.⁸

Interleukin-1 stimulates the phosphorylation and degradation of I κ B and activation of NF- κ B in corneal fibroblasts.⁹ Pro-IL-1 β is produced as

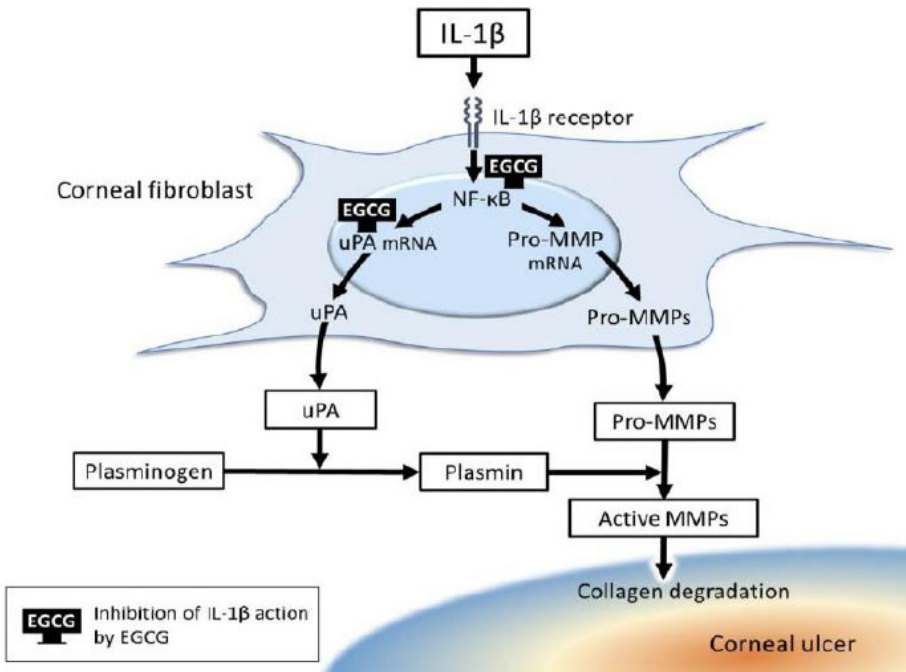


Figure 2. Inhibition mechanism by EGCG against collagen degradation stimulated by IL-1 β and corneal fibroblast.⁸

an inactive biologic pro-peptide that requires activation by caspase 1 through inflammasome activation to activate the cytokine. The inflammasome is a multiprotein signaling complex part of the innate immune system and plays a crucial role in activating the inflammatory response in the cornea and other organs.

Matrix Metalloproteinase (MMP)-9

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes involved in the pathophysiology of various ocular surface diseases.¹⁰ Cellular changes affect tissue remodeling, epithelial migration, and vascular proliferation. The cellular signaling pathways leading to MMP-9 expression are complex and involve multiple molecular interactions. One of the influencing factors is the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), a nuclear transcription factor known to bind to MMP-9 DNA promoter sites to increase MMP-9 mRNA levels. Inhibition of NF- κ B has shown decreased transcriptional activity of MMP-9 and improved growth of limbal epithelial cells following inflammation or injury to the ocular surface.¹¹

In several *in vitro* and *in vivo* animal studies previously conducted, the expression of MMP-9 after corneal

injury through mechanical spittle debridement, anterior keratectomy, and lamellar keratectomy showed a significant increase in MMP-9 levels in tears starting from 16 hours after injury up to 7 days. Normalization of MMP-9 levels occurred 7 days after injury and returned to basal levels 21-28 days after injury.¹¹

NF- κ B, IL-1, TGF- β , and PAF are proinflammatory mediators that increase MMP-9 expression in response to injury to the ocular surface, chemical injury, and infection.

Epigallocatechin gallate

Epigallocatechin gallate (EGCG) is the most abundant catechin in tea and has been shown to have anti-inflammatory and antioxidant effects on various types of cells. Due to its polyphenolic structure, EGCG can bind to proteins and nucleic acids more strongly than other green tea extracts. This binding is related to its structural and functional properties. Because of its unique properties, EGCG has therapeutic potential for various inflammatory diseases such as atherosclerosis, arthritis, dry eye disease, and various eye diseases.¹²

Previous studies have demonstrated various effects of EGCG on inflammatory pathways, burn and alkali injury to the

cornea, and corneal neovascularization (13–15). The optimal concentration of EGCG determined through various experiments was 20 μ g/mL based on toxicity and anti-inflammatory effects. This concentration was added to the buffer solution to create eye drops for the animal study.¹⁸⁻²¹

The inhibitory target of EGCG on collagen degradation is uPA rather than MMP.^{8,9} In the study conducted by Koji Sugioka, the effect of EGCG in inhibiting IL-1 β was found to be concentration-dependent. Based on the study results, significant concentrations were obtained at 30, 100, and 300 μ M.⁹ In another study conducted on human corneal epithelial cell cultures, EGCG at doses of 3-30 μ M was found to significantly inhibit the phosphorylation of transcriptional activity of MAPKs p38, JNK, NF- κ B, and AP-1.¹

In the degradation of corneal collagen by fibroblasts, the conversion of plasminogen to plasmin by uPA and activation of pro-MMPs by plasmin are the main pathways involved. EGCG inhibits the activation of pro-MMP1 produced by corneal fibroblasts in response to IL-1 β stimulation, and this effect is mediated by the inhibition of uPA upregulation without significant effects on pro-MMP1 expression. EGCG is known to inhibit collagen production or degradation by various types of cells. In hepatocyte cultures, EGCG suppresses collagen production and collagenase activation.¹⁶⁻²² EGCG also reduces the regulation of MMP3, MMP8, and MMP9 in gingival macrophage and fibroblast cultures.¹⁷⁻²² EGCG suppresses collagenolytic activity but does not affect MMP1 production in 3D corneal fibroblast cultures.⁹

The expression of the uPA gene is regulated by NF- κ B, with the gene promoter known to have a binding site for NF- κ B. EGCG inhibits the activation of NF- κ B and the increase of uPA mRNA in IL-1 β -stimulated corneal fibroblasts.⁹ EGCG does not significantly impair the pro-production of MMP1 by these cells but rather suppresses the upregulation of uPA, conversion of plasminogen to plasmin, and activating pro-MMP1 in response to IL-1 β stimulation. This inhibitory effect on uPA expression is associated with weakened activation of

NF- κ B. Considering that NF- κ B is also believed to enhance the presentation of pro-MMP1.⁹

This literature study has compiled some knowledges about the effect of EGCG in keratitis as an alternative adjuvant therapy. However, some questions regarding the significance and different outcomes in the expression of IL-1b and MMP-9 still need to be confirmed in future studies.

CONCLUSION

Epigallocatechin gallate (EGCG) in *Camellia sinensis* extract has the potential as an alternative adjuvant therapy for bacterial keratitis because it has antibacterial, anti-inflammatory and antioxidant effects.

CONFLICT OF INTEREST

There is no potential conflict of interest regarding this literature review study.

ETHICAL CLEARANCE

Not applicable for literature review.

FUNDING

This study was funded by the Ministry of Research, Technology and Higher Education Indonesia.

AUTHOR CONTRIBUTION

All authors have made the same contribution in writing the report on the results of this literature review

REFERENCES

- Cavet ME, Harrington KL, Vollmer TR, Ward KW, Zhang JZ. Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. *Mol Vis*. 2011;17:533-542.
- Swamynathan SK, Wells A. Conjunctival goblet cells: Ocular surface functions, disorders that affect them, and the potential for their regeneration. *Ocul Surf*. 2020;18(1):19-26.
- Grossniklaus HE. Ophthalmic Pathology: History, Accomplishments, Challenges, and Goals. *Ophthalmology*. 2015;122(8):1539-1542.
- Kamil S, Mohan RR. Corneal stromal wound healing: Major regulators and therapeutic targets. *Ocul Surf*. 2021;19:290-306.
- Weisenthal RW, Orlin SE, Daly MK, Tu EY, Freitas D de, Van Meter WS, et al. Infectious Diseases of the External Eye: Microbial and Parasitic Infections. In: Basic and Clinical Science Course 2020-2021 Section 8 External Disease and Cornea. American Academy of Ophthalmology; 2020:266-272.
- Eby AM, Hazlett LD. Pseudomonas Keratitis, a Review of Where We've Been and What Lies Ahead. *J Microb Biochem Technol*. 2016;8(1):9-13.
- Taube MA, del Mar Cendra M, Elsahn A, Christodoulides M, Hossain P. Pattern recognition receptors in microbial keratitis. *Eye*. 2015;29(11):1399-1415.
- Nishida T, Sugioka K, Fukuda K, Murakami J. Pivotal Role of Corneal Fibroblasts in Progression to Corneal Ulcer in Bacterial Keratitis. *Int J Mol Sci*. 2021;22(16):8979.
- Sugioka K, Yoshida K, Murakami J, Itahashi M, Mishima H, Nishida T, et al. Inhibition by Epigallocatechin Gallate of IL-1-Induced Urokinase-Type Plasminogen Activator Expression and Collagen Degradation by Corneal Fibroblasts. *Invest Ophthalmol Vis Sci*. 2019;60(8):2895-2903.
- Kaufman HE. The practical detection of mmp-9 diagnoses ocular surface disease and may help prevent its complications. *Cornea*. 2013;32(2):211-216.
- Jamerson EC, Elhusseiny AM, ElSheikh RH, Eleiwa TK, El Sayed YM. Role of Matrix Metalloproteinase 9 in Ocular Surface Disorders. *Eye & Contact Lens: Science & Clinical Practice*. 2020;46(2):S57-S63.
- Jin Y, Xi C, Qin J, Preedy VR, Yong J. Molecular Pathways, Green Tea Extract, (-)-Epigallocatechin Gallate, and Ocular Tissue. In: *Handbook of Nutrition, Diet and the Eye*. Elsevier; 2014:557-566.
- Gulias-Cañizo R, Lagunes-Guillén A, González-Robles A, Sánchez-Guzmán E, Castro-Muñozledo F. (-)-Epigallocatechin-3-gallate, reduces corneal damage secondary from experimental grade II alkali burns in mice. *Burns*. 2019;45(2):398-412.
- Koh CH, Lee HS, Chung SK. Effect of Topical Epigallocatechin Gallate on Corneal Neovascularization in Rabbits. *Cornea*. 2014;33(5):527-532.
- Lee HS, Chauhan SK, Okanobo A, Nallasamy N, Dana R. Therapeutic Efficacy of Topical Epigallocatechin Gallate in Murine Dry Eye. *Cornea*. 2011;30(12):1465-1472.
- Nakamuta M, Higashi N, Kohjima M, Fukushima M, Ohta S, Kotoh K, et al. Epigallocatechin-3-gallate, a polyphenol component of green tea, suppresses both collagen production and collagenase activity in hepatic stellate cells. *Int J Mol Med*. 2005;16(4):677-681.
- Morin MP, Grenier D. Regulation of matrix metalloproteinase secretion by green tea catechins in a three-dimensional co-culture model of macrophages and gingival fibroblasts. *Archives of Oral Biology*. 2017;75:89-99.
- Yang D, Cao G, Ba X, Jiang H. Epigallocatechin-3-O-gallate promotes extracellular matrix and inhibits inflammation in IL-1 β stimulated chondrocytes by the PTEN/miRNA-29b pathway. *Pharm Biol*. 2022;60(1):589-599.
- Chaurasia SS, Lim RR, Lakshminarayanan R, Mohan RR. Nanomedicine approaches for corneal diseases. *J Funct Biomater*. 2015;6(2):277-298.
- Fadhilina AN, Indriaswati L, Nurwasid, Fauziah D, Mahmudah. Expression of Superoxide Dismutase-1 (SOD-1) and changes of corneal endothelial morphology after phacoemulsification with hypothermic perfusion. *Bali Medical Journal*. 2022;11(3):1521-1526.
- Fariz M, Indriaswati L, Sutjipto. Effect of medroxyprogesterone and doxycycline on vascular endothelial growth factor (VEGF) expression and corneal neovascularization in corneal alkali trauma. *Bali Medical Journal*. 2023;12(2):1377-1380.
- Juwita A, Agustini L, Loebis R, Zuhria I, Ridholia, Ardianto C. Efficacy of topical medroxyprogesterone acetate 1 % and topical doxycycline reduce MMP-9 and TGF- β 1 expression as corneal ulceration due to alkali burn: literature review. *Bali Medical Journal*. 2023;12(2):1421-1425.



This work is licensed under a Creative Commons Attribution