INTRODUCTION

Pterygium is a prevalent pathological condition characterised by the proliferation of fibrovascular tissue and the development of wing-shaped conjunctiva on the corneal surface. The global recurrence rate of pterygium is high, ranging from 10% to 80%. In the specific case of Indonesia, the recurrence rate is reported to be between 24% and 89%. The persistent occurrence and advancement of recurrent pterygium pose an ongoing clinical dilemma for ophthalmologists in Indonesia, which remains unresolved. The presence of pterygium can lead to aesthetic concerns and has the capacity to induce a decline in visual acuity in its mature stages. This decline can be attributed to chronic inflammation, irregular astigmatism, closure of the optical axis, and hindrance of eye movement. Consequently, individuals affected by pterygium may necessitate many surgical interventions.1,3

Basic Fibroblast Growth Factor (bFGF) is a growth factor that is present throughout the phase of chronic inflammation and DNA damage. It has a crucial role in various cellular processes including cell migration, differentiation, proliferation, apoptosis, and angiogenesis. These processes collectively contribute to the invasion of pterygium cells. The induction of Basic Fibroblast Growth Factor (bFGF) has been seen in response to several stimuli, including ultraviolet (UV) light exposure. Apoptosis is a cellular mechanism characterised by programmed cell death, which has been observed to be closely linked to the development of pterygium. The presence of fibrosis is closely linked to the development of pterygium, as an elevated level of fibrosis is directly proportional to an elevated likelihood of pterygium recurrence.4,5

In the event of a reduction in fibrosis, the potential for inhibition of pterygium development arises. The mechanism under consideration is associated with apoptosis-related proteins, namely survivin, Bcl-2, Bax, and Bcl-w, which

ABSTRACT

Background: Pterygium is a prevalent ocular health issue. The efficacy of surgical procedures as the primary therapeutic approach has been limited, particularly in preventing its recurrence. Basic Fibroblast Growth Factor (bFGF) plays a significant role in the progression of chronic inflammation and DNA damage, leading to the invasion of pterygium cells. Epigallocatechin-3-gallate (EGCG) demonstrated the ability to decrease recurrence of human pterygium fibroblast (HPF) and inhibit cell migration in vitro, while preserving the integrity of conjunctival cells. This study aimed to examine the impact of EGCG on the expression of bFGF and cell apoptosis in HPF.

Methods: The experimental study with a randomized posttest only control group design was used to examine the expression of bFGF and cell apoptosis on HPF across 5 treatment groups. These groups included a control group (-), treatment groups receiving Mytomicin-C 0.4 mg/ml, as well as EGCG 50 µM, 100 µM, and 150 µM. Cell apoptosis was assessed by a flow cytometry instrument, while bFGF was accomplished by immunofluorescence staining.

Results: The results showed a significant decrease in bFGF expression in HPF cells among the control group (-), Mitomycin-C group and EGCG 50, 100, 150 µM group (p<0.01). The mean apoptosis induction of HPF cells after 72 hours in the control group, EGCG 50, 100, and 150 µM and MMC 0.4 mg/ml were respectively 6.1 (SD±0.08); 4.63 (SD±0.91); 20.70 (SD±1.46); 16.20 (SD±0.47); 98.81 (SD±0.16). ANOVA and Tukey posthoc tests showed a significant increase in apoptosis of HPF cells among treatment groups: the control group (-), EGCG 50, 100, and 150 µM and MMC 0.4 mg/ml were respectively 6.1 (SD±0.08); 4.63 (SD±0.91); 20.70 (SD±1.46); 16.20 (SD±0.47); 98.81 (SD±0.16). ANOVA and Tukey posthoc tests showed a significant increase in apoptosis of HPF cells among treatment groups: the control group (-), EGCG 50,100, 150 µM, and MMC 0.4 mg/ml (p<0.01), whereas EGCG 50 caused a decrease of apoptosis compared to control group (-).

Conclusion: There is a significant difference in the expression of bFGF and apoptosis in HPF after administration of EGCG compared to negative controls.

Keywords: EGCG, bFGF, cell apoptosis, Mitomycin.C.
play a role in the regulation of cellular death. The investigation of the expression of basic Fibroblast Growth Factor (bFGF) and apoptosis in Human Pterygium Fibroblasts (HPF) has been limited in its scope thus far. The primary treatment modalities employed in current surgical techniques are the conjunctival flap, conjunctival autograft, bare sclera, and the utilisation of amniotic membrane.

The efficacy of these diverse strategies in addressing the problem, particularly in terms of recurrence prevention, remains incomplete. The utilisation of primary therapy in conjunction with adjuvants, such as epigallocatechin-3-gallate (EGCG) found in green tea extract, exhibits considerable potential for therapeutic applications. Green tea, scientifically known as *Carmellia sinensis*, is recognised for its substantial content of catechin. This particular compound has been found to possess beneficial properties in the field of eye care, specifically as an anti-inflammatory, anti-inflammatory, and anti-oxidant agent. Epigallocatechin-3-gallate (EGCG) has demonstrated potential for utilisation in several formulations, including ophthalmic solutions.

Multiple studies have reported that the administration of EGCG at a concentration of 25 μM resulted in a significant reduction in pterygium cell proliferation by 16.78% (p<0.001) and 24.09% (p<0.001), as well as a notable inhibition of pterygium cell migration by 35.22% (p<0.001) and 25.20% (p=0.019) respectively. The administration of Epigallocatechin-3-gallate (EGCG) has demonstrated the ability to decrease the rates of pterygium fibroblast cell recurrence and cell migration in vitro, while not causing harm to conjunctival cells. As a result, the use of EGCG as an adjuvant therapy for primary pterygium treatment is proposed.

The presence of myocilin acid and mycophenolic acid in pterygium instances has been seen to diminish the expression of basic fibroblast growth factor (bFGF) during the wound healing process. This reduction in bFGF expression effectively hinders the proliferation of fibroblast cells. There is a possible impact of EGCG on the downregulation of basic Fibroblast Growth Factor (bFGF) expression and apoptosis in Human Pterygium Fibroblasts (HPF). Hence, the incorporation of EGCG as a supplementary agent, characterised by its minimum adverse effects, is anticipated to represent the optimal approach in mitigating the likelihood of pterygium recurrence in subsequent instances.

This study aimed to examine the impact of EGCG administration on the expression of bFGF and the occurrence of cell apoptosis in HPF, in comparison to the MMC and control groups.

**MATERIAL AND METHODS**

The study was a true experimental investigation employing human pterygium fibroblast cell culture obtained from human pterygium tissue. Human pterygium fibroblast cells were cultivated in a growth medium, followed by the administration of EGCG and MMC drops as per the findings of the optimal dosage treatment (Figure 1).

This study was a true experimental in vitro study using a research design with a randomized control group post-test only design, to determine the effect of EGCG according to the optimal dose test results and bFGF expression and cell apoptosis from pterygium fibroblast cells. The research will be conducted at the biomedical laboratories of the Faculty of Medicine at Brawijaya University, Malang, as well as the laboratory of the Faculty of Pharmacy at Airlangga University (UNAIR), Surabaya.

The study was conducted in September-November 2022. Inclusion criteria consists of pterygium patient of grade 3-4, the pterygium condition in the patients is characterised by a thick, fleshy appearance and a high degree of vascularity, the patients had previously undergone primary excision of the pterygium, the maximum age of the participants in the study is limited to 50 years. Exclusion criteria are patients with pterygium who exhibit characteristics of thinness, atrophy, and poor vascularization; the patient had undergone prior intraocular surgery; the abnormalities are present on the surface of the patient’s ocular globe.

The instruments employed in this study encompassed a range of tools, including sterile gloves, scissors, sterile tweezers, a culture dish, a 24-well microplate, a 96-well microplate, an incubator, a test tube, a centrifuge tube, a centrifuge, a laminar flow system, syringe filters, and sterilisers. The tools employed for analysing the examination results include of flow cytometry and image J software. The materials required for this research include a 0.93% sodium chloride (NaCl) solution, pure epigallocatechin gallate (EGCG), Hanks solution, red blood cell (RBC) lysis buffer, human basic fibroblast growth factor (bFGF) antibody, phosphate-buffered saline (PBS), penicillin, cell counting kit-8 (CCK-8) assay, streptomycin, propidium iodide (PI), Dulbecco’s Modified Eagle Medium (DMEM), 15% foetal bovine serum (FBS), bovine serum albumin (BSA), annexin V-fluorescein isothiocyanate (FITC), 4',6-diamidino-2-phenylindole (DAPI), vimentin antibody, formaldehyde, and 0.25% trypsin.

![Figure 1](image). The examination of bFGF expression and HPF cell apoptosis.
The Excision Procedure of Pterygium Tissue

The surgical procedure of pterygium excision was conducted in a sterile manner, employing the bare sclera technique. Subsequently, a sterile cloth is positioned within the surgical region, followed by the application of 10% povidone iodine to disinfect the eyeball and 5% povidone iodine to cleanse the surface of the eyeball. This is then followed by rinsing with Balanced Salt Solution (BSS). Prior to the pterygium tissue excision surgery, a subconjunctival injection of 2% lidocaine was administered to induce local anaesthesia.

The Isolation and Cultivation of HPF Cells

The sample was obtained from the apex of the pterygium, which was surgically removed around 2 mm from its border. Subsequently, the specimen was divided into multiple small fragments measuring less than 0.5 mm. These fragments were then rinsed with phosphate-buffered saline (PBS) and treated with a red blood cell (RBC) lysis buffer to induce lysis of erythrocytes. Following a 5-minute incubation period, the fragments were placed onto culture dishes with a diameter of 100 mm. The specimen was afterwards treated with 1 ml of DMEM solution comprising 15% FBS and Penicillin Streptomycin. It was then incubated overnight at a temperature of 37°C, with a humidity level of 95% and a gas mixture consisting of 5% CO2 and 95% air, while maintaining a humidity level of 95%. The culture medium, along with foetal bovine serum (FBS), was replenished on a daily basis. Once the cells have achieved 80% confluency, the concentration of foetal bovine serum (FBS), was decreased to 10% to provide sufficient time for the cells to adhere to the bottom surface of the plate. Each growth medium was administered to the specified test dose. HPF was tested at concentrations of 25–50–100–150 µM on growth media. Complete DMEM media was added to dilute EGCG at this concentration, then sonicated and filtered.

The Production of EGCG

Pure EGCG with a molar mass of 458.372 g/mol was dissolved with water to make a solution with a concentration according to the specified test dose. HPF was tested at concentrations of 25–50–100–150 µM on growth media. Complete DMEM media was added to dilute EGCG at this concentration, then sonicated and filtered.

The CCK-8 Assay and IC50 EGCG

The confluent cells were collected and quantified on a 96-well plate, with each well containing a total of 5000 cells. Once the cells have been carefully positioned onto the wellplate, it is advisable to provide sufficient time for the cells to adhere to the bottom surface of the plate. Each growth medium was administered EGCG at varying doses of 25, 50, 100, and 150 µM. The Cell Counting Kit (CCK) reagent was diluted with 50mg/10mL of phosphate-buffered saline (PBS) and afterwards dissolved in a 1:10 ratio with complete medium. A volume of 100 µL of this solution was applied to each wellplate, followed by an incubation period of 4 hours. Following the formation of crystals, the reagent was treated with dimethyl sulfoxide (DMSO) stopper reagent and subsequently incubated for a duration of 30 minutes. Subsequently, the cells were analysed using a microplate reader set at a wavelength of 450 nm. The data is given in a tabulated format, displaying the corresponding standard deviation. Subsequently, the cell viability and IC-50 dose are assessed in order to identify the most effective dosage of EGCG.

Data Analysis

All descriptive data from HPF criteria were analyzed by binomial test and presented as mean ± standard deviation. Data normality was tested using the Shapiro-Wilk test. Comparative data between bFGF expression and fibroblast apoptosis in 5 type interventions test (control, MMC and EGCG) was analysed using one-way Anova if the data is normally distributed and using the Kruskal-Wallis test if the data is not normally distributed. Then, if the results of the Anova test was significant, a PostHoc Tukey test is carried out to determine significant differences between 2 groups. The p value is considered significant if the p value is < 0.01. All statistical data were processed using SPSS software.

RESULTS

The Quantification of Cellular Apoptosis by Flow Cytometry

Each well plate was incubated for 72 hours. Subsequently, the sample was transferred into a centrifuge tube with a maximum capacity of 1.5 ml. It underwent three rounds of washing using cold phosphate-buffered saline (PBS) and binding buffer. Subsequently, the sample was subjected to centrifugation for a duration of 5 minutes. Subsequently, the cells were subjected to labelling using Annexin V-FITC and PI (Apoptosis kit Biolegend), a technique employed for the identification of cellular apoptosis, encompassing both early and late stages of the process. The fibroblast pterygium tissue, which is situated within the first tube, is held in a state of suspension within the binding buffer. Subsequently, a volume of 5 µl of Annexin V-FITC MTT was introduced into the tube that had undergone a 10-minute incubation period, and this was followed by the addition of 5 µl of PI (Propium Iodide). The samples were thereafter subjected to a 15-minute incubation period and were afterwards
analysed with flow cytometry equipment along with the accompanying analytic software provided by the same company.

The quantification of basic fibroblast growth factor (bFGF) was performed using immunofluorescence labelling. Confluent cells (P3-P7) are collected and the cell count is determined to be 25,000 cells per well plate. These cells are subsequently seeded into well plate 24 and allowed to grow till reaching confluence during a period of 24 to 72 hours. The well plate was partitioned into three groups: the control group, MMC 0.4 mg/mL group, and EGCG group. The control group only consists of cells that are cultivated in a growth medium. The cells were treated with an administration of MMC 0.4 mg/mL for a duration of 5 minutes. Subsequently, the cells were rinsed once with phosphate-buffered saline (PBS). The administration of EGCG is administered based on the dosage determined from the optimal and medium dose tests. Subsequently, a solution containing 3-4% formaldehyde was introduced into each well for a period of 15 minutes. Following this, the wells were rinsed twice with phosphate-buffered saline (PBS), thereby preparing the cells for examination utilising immunocytochemical techniques.

The subsequent phase, the wellplate underwent a series of washing steps using phosphate-buffered saline (PBS). Specifically, it was washed three times for a duration of 5 minutes each. Following this, the wellplate was subjected to an additional wash using PBS Triton-X 100 0.1% for a length of 5 minutes. Subsequently, the wellplate was incubated at room temperature for a period of 30 minutes using a solution containing 1% bovine sodium albumin (BSA). The liquid was disposed of and afterwards subjected to incubation with the primary antibody bFGF Rabbit Polyclonal Antibody Bioss bs-0431R® at a temperature of 4 degrees Celsius for a duration of 24 hours.

The antibodies underwent three washes with phosphate-buffered saline (PBS) for a duration of 5 minutes each. Subsequently, they were subjected to incubation with the secondary antibody bFGF at a dilution of 1:1000, at ambient temperature, for a period of 30 minutes. In the subsequent step, the wellplate underwent three rounds of washing with PBS for a duration of 5 minutes each. Following this, the wellplate was subjected to incubation with DAPI at a dilution of 1:1000 for a length of 5 minutes. Subsequently, the wellplate was washed again with PBS for a duration of 5 minutes. The results were observed and recorded utilising a fluorescent microscope with a magnification of 10x (Figure 2).

The analysis of expression levels was conducted utilising the ImageJ software. The results were quantified in terms of corrected total cell fluorescence (CTCF), which was calculated by subtracting the product of the area of the selected cell and the mean fluorescence of background readings from the integrated density.

**Human Pterygium Fibroblast Cell Viability Test with EGCG and MMC Intervention**

The CCK-8 assay conducted on EGCG demonstrated a notable decrease in cell viability, with statistically significant variations observed at each time point in comparison to the control group (Table 1). Doses ranging from 100 to 150 administered at the 72-hour mark are indicative of the threshold at which suppression of human pterygium fibroblasts is most pronounced. As a result, these specific doses are maintained throughout the intervention. The CCK-8 assay was conducted to evaluate the suppressive effect of mitomycin-C as a positive control. The results indicated a significant decrease in cell viability compared to the negative control group at the 72-hour time point. This effect was further observed throughout the intervention period (Figure 3).

The CCK-8 Assay and IC50 EGCG Test

The CCK-8 assay revealed a notable decrease in cell viability, with statistically significant variations observed across different doses and time intervals. The
administration of EGCG at concentrations of 25, 50, 100, 150 µM, and MMC at a concentration of 0.4 mg/ml resulted in a significant decrease in the number of high-power field (HPF) cells when compared to the control group. Specifically, at 24 hours, the reductions were 90.97%, 79.14%, 82.75%, 75.90%, and 78.21% respectively. At 48 hours, the reductions were 86.29%, 75.36%, 67.84%, 60.10%, and 47.05%. Finally, at 72 hours, the reductions were 67.68%, 61.99%, 49.27%, 10.65%, and 30.67% (Figure 3).

The IC50 value of EGCG is obtained from the equation $y = ax + b$ from the linear regression test of the percentage of inhibition for each observation time, where $y$ is 50 and $x$ is the IC50 of 104.65 µM (equivalent to 47.3 ppm) at 72 hours. By knowing the ability of EGCG to inhibit cells by 50% at a dose of 104.65 µM, it can be determined that doses of 100 and 150 µM are the desired doses for further intervention.

The inhibitory effect in the graph is found to be linear at all doses and observation times, so that EGCG can be concluded to be concentration-dependent and time-dependent. These results also show that EGCG is better compared to Mitomycin-C because the dose used is smaller based on the equation used (47.3 ppm vs 289.85 ppm) (Figure 4).

The average expression level of bFGF in the groups treated with EGCG at concentrations of 50 µM, 100 µM, and 150 µM exhibited a drop in comparison to the control group. This decrease in bFGF expression was seen to be inversely proportional to the concentration of EGCG administered, indicating that greater concentrations of EGCG resulted in lower levels of bFGF expression (Table 1).

The diagram presented in Figure 5 illustrates the visual representation of the data or concept being discussed. Nevertheless, the mean basic fibroblast growth factor (bFGF) expression in the MMC group exhibited the lowest value in comparison to the treatment groups.

The ANOVA test yielded statistically significant differences among the control group, the Mitomycin-C 0.4 mg/ml group, and the EGCG 50 µM, 100 µM, and 150 µM groups. The control group (-) had the

### Table 1. Average expression of bFGF

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean (pixel)</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>35.697.824</td>
<td>9.623.721</td>
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<tr>
<td>EGCG 50uM</td>
<td>9</td>
<td>11.588.699</td>
<td>1.876.256</td>
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<tr>
<td>EGCG 100uM</td>
<td>9</td>
<td>11.152.667</td>
<td>2.458.096</td>
<td>0.001**</td>
</tr>
<tr>
<td>EGCG 150 uM</td>
<td>9</td>
<td>6.302.796</td>
<td>892.658</td>
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</tr>
<tr>
<td>MMC</td>
<td>9</td>
<td>3.528.259</td>
<td>1.422.707</td>
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</table>

**ANOVA test, significant at $p < 0.01$
highest average bFGF expression, while the MMC 0.4 mg/ml group displayed the lowest average expression. The control group (-) yielded the highest result, whereas the MMC 0.4 mg/ml group yielded the lowest value. A posthoc test using the Tukey method (Table 5.4) revealed statistically significant variations in bFGF expression in HPF cells between the control group and the groups treated with different concentrations of EGCG (50 µM, 100 µM, and 150 µM) and MMC (0.4 mg/ml).

The study findings revealed a statistically significant distinction (p<0.01) in HPF cells among the group treated with 0.4 mg/ml MMC and the groups treated with EGCG at concentrations of 50 µM, 100 µM, and 150 µM. A statistically significant difference (p<0.01) was seen between the EGCG 50 µM group and the EGCG 100 µM and EGCG 150 µM groups (Table 1).

The study demonstrates that the treatment of 0.4 mg/ml MMC results in a significant reduction in bFGF expression in HPF cells as compared to the control group (-). Similarly, the inclusion of epigallocatechin gallate (EGCG) resulted in a decrease in basic fibroblast growth factor (bFGF) expression in human pulmonary fibroblast (HPF) cells, as compared to the control group (-). Similarly, it was observed that in the groups treated with varying concentrations of EGCG, there existed an inverse relationship among the concentration of EGCG administered and the expression of bFGF in HPF cells.

The Effect of Epigallocatechin-3-Gallate (EGCG) and Mytomycin-C (MMC) on the Induction of Apoptosis in Human Pterygium Fibroblast Cells

The assessment of cell apoptosis was conducted via flow cytometry, as depicted in Figure 6. The analysis of fibroblast cell apoptosis will be conducted after a period of 72 hours. The average rate of apoptosis induction in HPF cells after a 72-hour period was measured in the control group, as well as in groups treated with different concentrations of EGCG (50 µM, 100 µM, and 150 µM) and MMC (0.4 mg/ml).

The results are as follows: The data points 6.1 (SD±0.08), 4.63 (SD±0.91), 20.70 (SD±1.46), 16.20 (SD±0.47), and 98.81 (SD±0.16) were subjected to analysis by the utilisation of flow cytometry.

According to the findings presented in Figure 6 the group administered with EGCG at a concentration of 50uM exhibited the lowest average induction of HPF cell apoptosis (mean 6.362 ± 1.998). Conversely, the group treated with MMC at a concentration of 0.4 mg/ml demonstrated the highest average induction of HPF cell apoptosis (mean 96.865 ± 2.927). The findings of the study indicate that the highest level of apoptosis was observed in the group treated with a concentration of 100uM EGCG (mean 15.618 ± 5.651). Conversely, the group treated with 50uM EGCG exhibited the lowest level of apoptosis. This finding demonstrates that EGCG has the ability to trigger programmed cell death, known as apoptosis, in fibroblast cells within the high-power field (HPF).

The analysis of variance (ANOVA) test revealed a statistically significant disparity in the levels of apoptosis among the experimental groups (p=0.001). A posthoc test utilising Tukey’s method revealed statistically significant variations in apoptosis levels among high-power field (HPF) cells across several treatment groups, specifically the control group (-) and those treated with EGCG at concentrations of 50 µM, 100 µM, and 150 µM, as well as MMC at a concentration of 0.4 mg/ml (p<0.01) (Table 2).

The experimental groups consisted of EGCG at concentrations of 50 µM, 100 µM, and 150 µM, as well as a control group (-) and a group treated with MMC.

Table 2. The comprehensive description of cellular apoptosis.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>p-value</th>
</tr>
</thead>
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<td>Control</td>
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<td>7.805</td>
<td>2.078</td>
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</tr>
<tr>
<td>MMC</td>
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<td>96.865</td>
<td>2.927</td>
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<tr>
<td>EGCG 50uM</td>
<td>6</td>
<td>6.362</td>
<td>1.998</td>
<td>0.001**</td>
</tr>
<tr>
<td>EGCG 100uM</td>
<td>6</td>
<td>15.618</td>
<td>5.651</td>
<td></td>
</tr>
<tr>
<td>EGCG 150 uM</td>
<td>6</td>
<td>14.432</td>
<td>2.081</td>
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</tbody>
</table>

**ANOVA test, significant at p<0.01
at a concentration of 0.4 mg/ml. Statistical analysis revealed a significant difference between these groups (p<0.01). The experimental groups consisted of EGCG at concentrations of 100 µM, 50 µM, and 150 µM, as well as a control group (-) and a group treated with MMC at a concentration of 0.4 mg/ml. Statistical analysis revealed a significant difference between the groups (p<0.01). The experimental groups consisted of EGCG at concentrations of 150 µM, 50 µM, and 100 µM, as well as a control group (-) and a group treated with MMC at a concentration of 0.4 mg/ml. Statistical analysis revealed a significant difference between these groups (p<0.01). The experimental setup included the use of MMC at a concentration of 0.4 mg/ml, along with control samples. Additionally, three different concentrations of EGCG (50 µM, 100 µM, and 150 µM) were tested. Statistical analysis revealed a significant difference between the groups (p<0.01).

**DISCUSSION**

The viability of HPF cells was assessed through the utilisation of the CCK-8 Assay, which revealed a decrease in results subsequent to the administration of EGCG. This finding demonstrates that EGCG exhibits inhibitory effects on the proliferation of HPF cells in vitro, suggesting its potential function in preventing the recurrence of pterygium. Epigallocatechin gallate (EGCG) has a significant role in the inhibition of Reactive Oxygen Species (ROS) creation or ROS-induced damage. This is achieved by lowering the production of NADPH - cytochrome P 450 at the C-1 position. The induction of cell cycle arrest at the G2/M phase by EGCG is mediated by the downregulation of cyclin D1 expression and the upregulation of CDK inhibitors, resulting in the deactivation of CDK and subsequent induction of cell death.10

Yang et al. (2014) examined the impact of EGCG on HPF cells. He administered EGCG doses of 25 and 50 µM to investigate their effects on HPF cell viability. The results indicated a significant reduction in HPF cell viability, with percentages of 24% and 29% seen on day 7, as compared to the control group. Several factors can influence the outcomes of the treatment, including variations in the type of EGCG utilized and the timing of the observation conducted.

The viability of HPF cells might be hindered by the administration of Mytomycin-C (MMC) at a concentration of 0.4 mg/ml. This treatment exhibited a noteworthy suppressive impact when compared to the control group.11

Epigallocatechin-3-Gallate (EGCG) is a potential adjuvant therapy that may effectively mitigate the recurrence rate of pterygium by its inhibitory effects on angiogenesis, proliferation, and induction of apoptosis in fibroblast cells. The investigation aimed to ascertain the most effective dosage and IC50 of EGCG for HPF cells. The experimental results revealed that a concentration of 104.65 µM administered over a duration of 72 hours yielded the desired outcome.

Wang et al. (2012) assert that mammalian cytosolic thioredoxin reductase (TrxR1) is an enzymatic entity known for its chemo-preventive properties against cancer. However, it is important to note that many of the inhibitors associated with TrxR1 exhibit deleterious consequences on human health. The inhibitory effect of EGCG green tea extract on TrxR1 has been established, as evidenced by its IC50 value of 256 microg/ml.12

Shirakami et al. (2018), stated that green tea catechins possess the ability to impede the multiplication of cancer cells. At a dosage of 50 µM, various catechin derivatives, namely gallo-catechin, epigallocatechin gallate, and gallocatechin gallate, have been identified as highly efficacious against colon cancer cells. Notably, gallo-catechin exhibits an effectiveness rate of 85%, while gallocatechin gallate has a remarkable efficacy rate of 93%. In the present study, it was observed that gallo-catechin exhibited a percentage of inhibition of 87% in lung cancer cells, while gallocatechin gallate demonstrated a percentage of inhibition of 67%.13

Tsuchiya et al. in 2020 stated the substances epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) exhibited an inhibitory effect on cell proliferation at a concentration of 100 µM. In comparison to the control variables, the EGCG compound exhibited a resistance to tumour cell culture of 34.8 ± 2.2% at 24 hours and 69.2 ± 1.1% at 72 hours, respectively.17

The administration of MMC as an adjuvant therapy during or post-surgery has been found to substantially decrease the recurrence rate of pterygium by inhibiting the proliferation of fibroblast cells. However, it is important to note that the utilisation of MMC is also associated with severe problems. Pterygium is known to suppress the proliferation of rapidly dividing cells, such as fibroblasts and vascular endothelial cells, in the episcleral region following excision. The utilisation of Mitomycin C (MMC) in pterygium surgery is not officially approved by the Food and Drug Administration (FDA), therefore falling under off-label usage. However, MMC is commonly employed in the management of cancer. Multiple investigations conducted on primary pterygium have established that the use of various quantities of intraoperative mitomycin C (MMC), ranging from 0.02% to 0.04%, delivered over a period of 3 to 5 minutes, resulted in a significant reduction in pterygium recurrence compared to the technique of excision with bare sclera resection (BSR). Hence, in order to mitigate the likelihood of recurrence, the majority of publications have recommended the administration of a solitary dose of mitomycin C (MMC) intraoperatively following pterygium excision.18

The administration of mitomycin C (MMC) at a concentration of 0.02% for a duration of 2 minutes is widely employed. Notably, no instances of severe problems associated with this dosage regimen have been documented. The intraoperative application of 0.04% MMC for a duration of 3 to 5 minutes may lead to a delay in epithelialization. However, there have been no documented cases of delayed epithelialization associated with the administration of 0.02% MMC for a duration of 3 minutes. The occurrence of iritis and dellen cornea has been documented in 3% of cases when a 0.01% concentration of mitomycin C (MMC) was administered for a duration of 5 minutes during surgical procedures. The most favourable dose of mitomycin C (MMC), the appropriate duration of exposure, and the ideal application site, namely the bare
The COX-2 enzyme plays a pivotal role in high-passaged fibroblast (HPF) cells. The involvement of COX-2 in the process of angiogenesis that is the vascular epithelium of pterygium. This finding is in line with the outcomes of the aforementioned investigation. Ferrari et al. (2022) stated EGCG possesses the capacity to impede cellular proliferation in an in vitro setting, including the inhibition of basic fibroblast growth factor (bFGF). The stability and bioavailability of EGCG polyphenols enable their penetration into the nucleus, where they can interact with DNA and RNA, thereby exerting a regulatory influence on gene expression. EGCG has been documented to have binding affinity towards a diverse array of 55 protein types, including kinases, growth factor receptors, apoptotic proteins, and the proteasome. Consequently, these interactions have the potential to impede cellular growth processes.

The presence of basic fibroblast growth factor, commonly referred to as FGF-2, is highly prevalent in pterygium fibroblast cells. The involvement of [the subject] encompasses angiogenesis, wound healing, numerous endocrine pathways, and the augmentation of invading mast cells within the vascular epithelium of pterygium. Detorakis et al. (2019) reported that basic fibroblast growth factor (bFGF) has the capacity to stimulate the upregulation of cyclooxygenase-2 (COX-2) expression, a protein that is not typically found in healthy conjunctival tissue but is observed in high-passaged fibroblast (HPF) cells. The COX-2 enzyme plays a pivotal role in the process of angiogenesis that is triggered by inflammatory cytokines. Consequently, basic fibroblast growth factor (bFGF) has the capability to produce both inflammation and angiogenesis in pterygium. In a recent study, it was observed that microphenolic acid has the ability to modify the expression of basic fibroblast growth factor (bFGF) in pterygium, hence exhibiting an inhibitory impact on the proliferation of fibroblast cells.

Kria, Ohira, and Amemiya (1998) found that the immunoreactivity of b-FGF was more pronounced in fibroblast cells of recurrent pterygium as compared to those of primary pterygium. The robust immunoreactivity and subsequent release of basic fibroblast growth factor (b-FGF) seen in cultured fibroblasts of recurrent pterygium indicate that fibroblasts have a significant involvement in the recurrence of pterygium.

Lin et al. (2020) revealed a noteworthy reduction in the vitality of Tenon fibroblast cells when exposed to a concentration of 100 µM EGCG, with statistical significance observed (p<0.05). Similarly, Yang et al. (2014) reported in their research findings that the application of EGCG at concentrations of 25 µM and 50 µM resulted in a significant inhibition of pterygium cells on the seventh day (p<0.01). One study, which reported that the administration of 40 microg/ml green tea extract (GTE) or EGCG resulted in a decrease in the levels of the angiogenic factor bFGF in cells, demonstrating an inhibitory effect on angiogenic fibroblast growth factor.

The substantial impact on the activity of the 20S proteasome. Specifically, it was seen that EGCG decreased the chymotrypsin-like and caspase-like activity, while increasing the trypsin-like activity. These effects were assessed using specific substrates. The findings presented in this study align with prior research, which has demonstrated that lactacystin possesses inhibitory qualities similar to trypsin, hence exerting an influence on the inhibition of basic fibroblast growth factor (bFGF) by epigallocatechin gallate (EGCG).

This study provides evidence supporting the potential efficacy of EGCG as a substitute for MMC in adjuvant therapy for reducing pterygium recurrence. This is attributed to its ability to hinder cell survival and migration, as well as diminish bFGF expression in HPF cells. This work additionally demonstrates that MMC continues to exhibit superior efficacy compared to EGCG in terms of suppressing cell survival and migration, as well as downregulating bFGF expression in HPF cell cultures. Nevertheless, it is crucial to acknowledge the potential efficacy of MMC in human subjects, while also taking into account the substantial incidence of problems that may emerge, thereby potentially compromising patient outcomes.

Elevated levels of apoptosis can lead to enhanced cellular proliferation in damaged cell populations. In the event that macrophages fail to adequately cleanse these cells, a state of persistent inflammation and fibrosis may ensue. The presence of resistance to apoptosis can lead to the development of fibrosis. Epigallocatechin-3-Gallate (EGCG) has the potential to inhibit the occurrence of this phenomenon from its early onset. The administration of Mytomycin-C (MMC) has the potential to trigger programmed cell death, known as apoptosis, in human pterygium fibroblast (HPF) cells. Mytomycin-C (MMC) is an anti-metabolite derived from Streptonicas caespitosus. It functions as an alkylating agent, impeding the production of DNA, RNA, and proteins. This disruption of cellular processes can induce cell apoptosis due to the inability to repair genotoxic damage. Additionally, MMC hinders cell division during the G1 phase and early stages of mitosis. The S phase of the cell cycle.

Mytomycin-C has been observed to induce metabolic activity through its ability to inhibit cytochrome P-450 enzymes and generate alkylating agents. The inhibition of DNA, RNA, and protein synthesis can subsequently induce cellular death due to the impaired ability to repair genotoxic damage, leading to a reduction in the occurrence of cell mitosis during the late G1 phase and early S phase of the cell cycle. Shi et al. (2013) align with the outcomes of the present investigation, which reported that the administration of 0.4 mg/ml of MMC for a duration of 5 minutes, followed by a 72-hour observation period on in vitro fibroblast...
cells, resulted in an enhanced induction of apoptosis in these cells.\textsuperscript{26} Mitomycin-C has the potential to decrease the presence of inflammatory cells, goblet cells, and fibroblast cells, while also exhibiting an anti-fibroblastic effect.\textsuperscript{27}

The study examined the impact of apoptosis induction in fibroblasts following treatment with a concentration of 0.4 mg/ml of MMC for a duration of 5 minutes, followed by triple washing with PBS. Subsequently, the fibroblasts were stained with Annexin FITC and subjected to a double staining PI test. Finally, flow cytometry was employed to analyse the obtained data. The experimental results revealed that MMC 0.4 mg/ml exhibited the most pronounced impact on the induction of cell apoptosis, surpassing both the control and EGCG groups. MMC has the ability to impede cellular proliferation, trigger apoptosis, enhance T-cell-mediated cell lysis, and diminish the production of pro-fibrosis genes. MMC has the ability to impede the proliferation of cells and induce apoptosis in fibroblast cells derived from patients with rheumatoid arthritis. The apoptotic process in this context can be mediated through the mitochondrial signalling system.\textsuperscript{28}

EGCG has the ability to trigger apoptosis in human pterygium fibroblast cells. The findings presented here are consistent with the study conducted by Hidayat et al. (2020), which reported that EGCG has the ability to suppress the production of anti-apoptotic proteins Bcl-1 and Bcl-xl, while simultaneously enhancing the expression of pro-apoptotic proteins Bax and Bak. This phenomenon is facilitated by multiple processes, specifically, the activation of caspase 3 and caspase 9, the regulation of mitochondrial function through circuit – 3, and the degradation of polyADP ribose polymerase activity. Cell apoptosis is the result of circuit disruption, namely circuit-3 disturbance. The interference of EGCG in cancer cells, including pterygium, occurs within the mitochondria, specifically targeting a gene known as sirtuin-3. The inability of sirtuin-3 to effectively neutralise all free radicals, coupled with the high abundance of reactive oxygen species (ROS) within mitochondria, leads to cellular death.\textsuperscript{28}

Sukhthankar et al (2008) stated that EGCG has the potential to serve as a therapeutic agent for impeding the growth of pterygium. This is achieved by suppressing the proliferation of fibroblast cells through the downregulation of bFGF expression, which is known to induce cell death via the apoptotic pathway. However, additional research is required in order to gain a more comprehensive understanding of the mechanism by which EGCG affects apoptosis in colorectal cancer. The present study also established that the basic fibroblast growth factor (bFGF) protein exhibited a quick degradation when exposed to epigallocatechin gallate (EGCG), thereby demonstrating its noteworthy role in the suppression of metastasis in colorectal cancer.\textsuperscript{29}

The antioxidant action of EGCG hinders the generation or harm induced by reactive oxygen species (ROS) originating from inflammation that underlies the development of pterygium. Epigallocatechin gallate (EGCG) has been found to decrease the levels of glutathione, leading to an elevation in reactive oxygen species (ROS) generation. Furthermore, epigallocatechin gallate (EGCG) has the ability to decrease the generation of (NADPH)-cytochrome P450 resulting from reactive oxygen species (ROS), hence exhibiting anti-inflammatory properties.\textsuperscript{1,13,28}

This study observed a notable rise in fibroblast cell death following the injection of Mitomycin-c and EGCG in high-power fields (HPF), with the exception of a reduction in fibroblast cell apoptosis at an EGCG dose of 50 µM as compared to the negative control group. The observed reduction in apoptosis in this study aligns with the concurrent decrease in apoptosis of healthy cells, thereby facilitating the proliferation and metastasis of cancerous cells. In contrast, it has been demonstrated that EGCG can impede specific signalling pathways that promote cellular survival and proliferation, hence potentially diminishing cell proliferation rates and promoting apoptosis in cancerous cells. In general, the impact of EGCG on apoptosis is contingent upon the particular circumstances and cancer cell types under investigation. The induction or suppression of apoptosis can be modulated by EGCG, contingent upon the specific contextual factors. In certain instances, epigallocatechin gallate (EGCG) has demonstrated the ability to initiate programmed cell death, known as apoptosis, in cancer cells. This mechanism has the potential to impede or halt the proliferation of tumours. Nevertheless, under alternative circumstances, EGCG has the capability to reduce apoptosis, so offering protection to healthy cells from potential harm or demise. EGCG has the potential to mitigate neuronal apoptosis, hence potentially safeguarding these cells against the detrimental effects and mortality associated with illnesses such as Alzheimer’s disease. Furthermore, it has been observed that EGCG exhibits the ability to mitigate apoptosis in the hepatic tissue, hence potentially offering a safeguard against hepatic injury induced by the use of alcohol or other harmful substances.\textsuperscript{29,30}

The findings of this study indicate that the treatment of 150 µM EGCG resulted in the most significant impact, leading to reduced expression of bFGF in HPF. Similarly, the administration of Mitomycin-c also resulted in reduced bFGF expression in HPF. The application of EGCG at a concentration of 100 µM resulted in the most pronounced apoptotic response in fibroblast cells within the high-power field (HPF). Similarly, the administration of Mitomycin-c also induced apoptosis in fibroblast cells within the HPF. The study’s findings indicate a notable increase in fibroblast cell death in the high-performance liquid chromatography (HPF) group following the administration of EGCG, in comparison to the negative control group. However, it is worth noting that at an EGCG concentration of 50 µM, fibroblast cell apoptosis was shown to be lower than that of the negative control group.

In general, the effect of EGCG on apoptosis exhibits intricacy and is contingent upon the particular cellular phenotype and the surrounding circumstances in which it is administered. Additional investigation is warranted in order to comprehensively comprehend the processes via which EGCG governs apoptosis and to ascertain the optimal applications of this substance in the...
treatment of diverse health disorders. The potential of EGCG as a substitute for MMC in causing apoptosis in fibroblast cells in HPF is encouraging. However, it is important to note that MMC still outperforms EGCG in terms of generating fibroblast cell death in HPF cell cultures. Further research is required to be conducted on a more extensive sample size, as well as with a higher dosage comparable to an MMC dose of 0.4 mg/ml, and a longer duration of observation.

One potential limitation of the study lies in its exclusive focus on examining the direct pathway by which EGCG impacts bFGF expression and death in fibroblast cells within the context of HPF. Additional investigation is required to examine the precise mechanistic links involved in the impact of EGCG intervention on the recurrence of pterygium. Further investigation is warranted to explore the intricate pharmacodynamic pathway of EGCG in future developmental investigations. Our investigation serves as a preliminary examination of the possible effects of EGCG on bFGF expression and fibroblast cell death in HPF, as adjuvant therapy of MMC.

CONCLUSION

There is a significant difference in bFGF expression in HPF after EGCG administration with a lower mean bFGF expression compared to the MMC and control group. There was a significant difference in fibroblast cell apoptosis in HPF after administration of EGCG with a higher mean apoptosis, except that at an EGCG concentration of 50 µM, fibroblast cell apoptosis was found to be lower compared to the MMC and control group because the IC50 effective dose was found to be 104.65. EGCG is better than MMC in reducing bFGF expression and inducing apoptosis based on IC50 dose balance.

CONFLICT OF INTEREST

The authors affirmed that there were no conflicts of interest in this study.

ETHICAL CLEARANCE

This study has obtained ethical clearance from Faculty of Medicine, University of Airlangga, Surabaya with reference letter number 166/EC/KEPK/FKUA/2022.

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AUTHOR CONTRIBUTION

All authors contributed equally in this research and publication of this manuscript.

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