Exploring the effect of epigallocatechin gallate on non-small cell lung cancer

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Abstract: Introduction: Human epidemiological studies have shown that diets rich in plant polyphenols have beneficial effects on various diseases including cancer. Epigallocatechin Gallate, a flavonoid polyphenol molecule, has been shown to be both chemotherapeutic and chemo-preventive in the treatment of several forms of cancer, including lung cancer. 80% of cancers of the lungs are non-small cell lung cancers. Objective: The study was carried out to see the effects of Epigallocatechin Gallate in non-small cell lung cancer cells (A549) using in-vitro studies. Materials and Methods: Cell Viability Assay was performed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Wound Healing assay was also performed at different concentrations of the compound. Dexamethasone and Doxorubicin, the drugs with anti-cancer properties served as control. A549 cell lines were used. Results: In the current study, it was demonstrated using Cell viability assay and Wound Healing assay that Epigallocatechin gallate exhibits anti-proliferative activity on A549 lung cancer cells and inhibits cancer cell proliferation in a concentration and time-dependent manner. It was observed that Epigallocatechin gallate (P = 0.0016, P = 0.0018) could significantly inhibit the growth of lung cancer cells with IC50 values 60.55 ± 1.0 μM. The result of wound Healing assay suggests that Epigallocatechin gallate can inhibit the proliferation and migration of A549 cells with concentrations near or higher to 50 μM. Conclusion: Epigallocatechin gallate’s protective effect has been shown in A549 lung adenocarcinoma cells in a time and dose-dependent manner. This suggests the implication of Epigallocatechin gallate for the prevention and therapy for lung cancer.

Keywords: Chemopreventive, lung cancer, natural products, epigallocatechin gallate, therapeutic

Introduction

For several decades, lung cancer has been the most frequent cancer worldwide. It is the third most frequent cancer but it accounts for the highest rate of all cancer-related deaths (22%) [1]. Lung carcinomas are classified into two types based on histotype, prognosis, and treatment implications: small-cell carcinoma (SCLC, 13% of cases) and non-small-cell carcinoma (NSCLC, 83% of cases) [2]. Approximately 85% of patients have a set of histological subtypes known as NSCLC; with around 40% being adenocarcinoma, 25 to 30% being squamous cell carcinoma, and 10 to 15% being large cell carcinoma [3, 4]. Lung cancer has been linked to several environmental and behavioral risk factors [5]. The clinical results in lung tumors are directly proportional to the cancer stage at the point of diagnosis. For instance, those suffering from stage I lung cancer have a five-year survival rate of 68.4%, while patients with stage IV lung carcinoma have a five-year survival rate of 5.8% [6].

The usual cancer treatments include surgery, chemotherapy, radiation, and, if applicable, immunotherapy. These can be given separately or in sequence. Several studies have employed Doxorubicin as a chemotherapeutic treatment for lung tumours [7]. Doxorubicin, an antibiotic, has been investigated for decades and is used as a chemotherapeutic treatment for lung cancer. However, Doxorubicin medication includes negative side effects such as cardiotoxicity and hair loss, among others [8]. Dexamethasone is a popular anti-cancer synthetic glucocorticoid. The anti-cancer activity of dexamethasone in solid tumours was recently shown [9]. It has the potential to reduce the viability of NSCLC
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Dexamethasone and Doxorubicin have been found to show anti-proliferative impact in several malignancies [10-12].

Several natural bioactive substances have recently been shown to have anticancer capabilities, capable of destroying altered or cancerous cells while remaining non-toxic to their normal cells. This impact happens when natural products are combined with conventional treatments, implying that nutraceutical supplementation may aid in successful anticancer therapy [13, 14]. There is substantial epidemiological evidence that a fruit and vegetable-rich diet may reduce the risk of various malignancies. Natural polyphenols are thought to contribute to the effect. Furthermore, multiple studies have shown that natural polyphenols can help prevent and treat cancer. Possible mechanisms include antioxidants, anti-inflammation, and regulation of various molecular events implicated in carcinogenesis [15].

Tea is drunk all over the world, and epidemiological and clinical research has demonstrated its health benefits, including anti-cancer properties. Green tea polyphenols are mostly composed of epigallocatechin gallate (EGCG), which is thought to be accountable for the majority of these effects [16]. EGCG is a polyphenolic substance found in green tea extract that has demonstrated a variety of bioactivities in preventing cancers of various kinds. EGCG has a number of biological roles, including anti-cancer and anti-inflammatory characteristics [17]. Research has shown how important EGCG is for the treatment and prevention of disease. Its anti-inflammatory and antioxidant qualities are responsible for its function in the management of illnesses. This green tea compound's anticancer effect in numerous cancer types has been verified, however it is still being investigated [18]. Cell culture and animal research, as well as epidemiological and clinical investigations, have all shown that EGCG has anti-tumoral properties [19]. Several clinical trials have shown that EGCG administration reduces tumor incidence and multiplicity in various organ sites, including the colon, liver, lung, skin, stomach, and mammary gland [20].

Several preclinical investigations have demonstrated that green tea and its contents play an essential role in the avoidance and cure of human carcinomas. A study of 49,920 men aged 40-69 years in Japan who completed a questionnaire that included consumption of green tea habits at baseline found that drinking 5 or more cups/day in comparison to less than 1 cup/day reduced the risk of advanced prostate cancer [21]. A randomised phase II clinical preventive research found that consuming 10 Japanese-size cups of green tea, combined with green tea tablets, significantly decreased tumour recurrence in individuals with colorectal cancers [22]. In another study, conducted over a ten-year period, Nakachi and Inami discovered a total of 419 cancer patients, 175 women and 244 men that drinking more than 10 cups of green tea per day (equivalent to 2.5 g green tea extract) also considerably reduced the risk of lung cancer, with a relative risk of 0.33, followed by colorectal, liver, and stomach cancers in this order [23].

In this work, the viability and migration rate of cells in A549 lung adenocarcinoma cell lines were evaluated in order to acquire a deeper understanding of EGCG's damaging effect on cancer cells. EGCG's IC50 (50% inhibitory concentration) was established in NSCLC cell lines utilising a cell viability experiment. The migration rate evaluated using the wound healing assay demonstrated the effects of EGCG in a time and concentration-dependent manner. EGCG has been found to cause cancer cell death and consequently limit migration.

Methodology

In-vitro A549 cell culture

In in-vitro conditions, A549 cells (human lung adenocarcinoma cells, National Center for Cell Sciences, Pune) were grown in Dulbecco's Modified Eagles Medium (DMEM) (Merck, D5648) supplemented with 10% Foetal Bovine Serum (FBS) (Merck, F7524), 3.7 g/L sodium bicarbonate (Merck, S5761), and 1% pen-strap antibiotic solution (Merck, P7531). The culture was kept in a CO₂ incubator with a constant supply of 5% CO₂ at 37°C.

Anti-proliferative activity

Cell viability assay (MTT assay): A549 cells were seeded on cell culture-treated 96-well plates at a density of 1 × 10⁴ cells per well. Following 24 hours of incubation, the culture was treated with different doses of EGCG.
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(Himedia, RM10179) (0-500 M in 0.3% DMSO) and incubated for an additional 48 hours at 37°C. After 48 hours of drug treatment, cell viability was assessed by adding MTT salt solution (Merck, M2003) (5 mg/mL in 1 x PBS) for 3 hours. Insoluble formazan crystals were dissolved in DMSO (Himedia, GRM5856), and absorbance was measured at 570 nm using a BioTek Synergy H1 hybrid multimode microplate reader (USA). Cells in control wells with no treatment showed 100% cellular proliferation. The IC50 value concentration of EGCG was computed using the GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, California, USA).

Wound healing assay: Wound healing assays provide a simple, inexpensive, and highly reliable approach for studying migration of cancer cells in vitro. It relies on the finding that cells in a monolayer migrate to reestablish cell connections following the formation of an artificial wound. The assay consists of creating a wound in a monolayer, acquiring images during wound closure, and comparing the migrated area at the initial and final time periods [24]. A549 cells (1 x 10^6 cells/well) were seeded on a 6-well plate for 48 hours to achieve confluent cells greater than 85%. The cells at confluence were then scraped proportionately with a 10 ul pipette tip. Cells were cultured in DMEM media conditioned with EGCG at various doses (500 μM, 250 μM, 100 μM, and 50 μM). The same protocol for wound healing assay has been followed in already reported studies [25]. Wells without any compounds were used as controls. The growth and migration of A549 cells were assessed by evaluating the time it took for the wound to heal following monolayer gap closure. Microscopy was done under the 20X magnification using Primovert, Zeiss microscope. ImageJ software, with the Axio cam’s built-in calibrated scale, recorded images of scratches at time intervals of 0 hour, 24 hours, 48 hours, and 72 hours. The wound closure rate was computed as follows:

Wound area closure rate (%) = (Area_{t=0h} - Area_{t=Δh}) / Area_{t=0h} × 100

Where, Area_{t=0h} is the area of the wound at zero hours of compound treatment, and Area_{t=Δh} is the area of the wound at ‘h’ hours of compound treatment.

The rate of cell migration was investigated by measuring the closest distance between cells within a designated gap in the wound region. The following calculation was used to analyze the cell migration rate (Rm):

Rate of cell migration (Rm) = (W_{t=0h} - W_{t=Δh}) / t

Where, W_{t=0h} is the width of the wound at 0 hour, W_{t=Δh} is the width of the wound at t hours, and t is the time duration of migration in hours.

Statistical analysis

All tests were performed in triplicate, and the IC50 value was determined using the triplicate results. GaphPad Prism 8.0.1 was used to analyze nonlinear regression and determine IC50 values. Microsoft Office Excel 2016 was used to export raw data.

Results

Cell viability assay: effect of EGCG on cancer cell viability

The present investigation revealed the presence of potent anti-proliferative property against A549 cells by compound EGCG (Figure 1). Further, the study with Dexamethasone and Doxorubicin as standard drugs, suggested that EGCG (P = 0.0016, P = 0.0018) could significantly inhibit the growth of lung cancer cells with IC50 values 60.55 ± 1.0 μM (Table 1). The study revealed the anti-proliferative potential of EGCG on A549 lung adenocarcinoma cells. As per the result of Tukey’s multiple comparison test (Table 2), EGCG showed significant results, when compared with the standard drugs. The compound inhibited the proliferation of A549 cells in a concentration dependent manner. As depicted in Table 1, EGCG had a much lower IC50 value as compared to one of the standard drugs, Dexamethasone where EGCG was found to be almost 3 times more potent than Dexamethasone implying that it’s anti-proliferative potential is much better than this standard drug, making it favorable for use in cancer treatment.

Wound healing assay: effect of EGCG on cancer cell proliferation

The wound healing assay has been used in various cancer studies to depict the role of differ-
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Table 1. IC50 values of anti-proliferative activity of test compounds on A549 lung adenocarcinoma cells

<table>
<thead>
<tr>
<th>Test Compounds</th>
<th>IC50 Values (μM)</th>
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<tbody>
<tr>
<td>Epigallocatechin gallate</td>
<td>60.55 ± 1.0</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>227.9 ± 3.7</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>3.9 ± 0.2</td>
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</tbody>
</table>

The values are represented in IC50 (μM) ± Standard error mean (S.E.M.), where n = 3.

Table 2. One way-ANOVA analysis of the tested compounds on variation of concentration

<table>
<thead>
<tr>
<th>Tukey’s multiple comparisons test</th>
<th>Significant?</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexa vs. EGCG</td>
<td>Yes</td>
<td>0.0016</td>
</tr>
<tr>
<td>Doxy vs. EGCG</td>
<td>Yes</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Different compounds impacting the migration rate of cancer cells. Compounds that decrease the rate of migration of cancer cells have anti-cancer properties [26-29]. The study of wound assays on treatment with the four different concentrations of 50, 100, 250, and 500 μM of EGCG revealed that the lung adenocarcinoma cells showed delayed growth rate and proliferation over time (Table 4). A549 cells migrated fast and closed the wound gap within 48 hours in experimental control (wound without compound treatment) (Figure 2). EGCG inhibited the growing cancer cells in concentration-dependent manner. In the wound, treated with 50 μM EGCG wound area that remained was 73.36% in 24 hours (Table 3). However, in a higher concentration of 500 μM wound area confluence remained up to 91.48% (Table 4). In control, 100% complete wound confluence was observed at 96 hours with 0 micrometer (μm) wound area. The rate of migration by A549 shows highest at 24 hours in control with 39.75 μm/h and decreases over time up to 72 hours. The migration rate in EGCG treatment revealed a lesser migration rate at a higher concentration of 500 μM, indicating the concentration-dependent behaviour of A549 cell growth (Table 5; Figure 3). The study revealed the presence of important anticancer properties shown by the EGCG on lung cancer cells that caused the inhibition of the growth of cancer cells and proliferation at very low concentrations with high efficacy.

Discussion

Lung cancer happens to be one of the most prevalent cancers in humans, with a high mor-
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Table 3. Percentage of wound area remained in time interval of 96 hours on treatment with four different concentrations of EGCG

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Percentage of wound area over time</th>
<th>Control</th>
<th>50</th>
<th>100</th>
<th>250</th>
<th>500</th>
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<tbody>
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<tr>
<td>24</td>
<td></td>
<td>17.56</td>
<td>73.36</td>
<td>79.91</td>
<td>86.55</td>
<td>91.48</td>
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<tr>
<td>48</td>
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<td>5.79</td>
<td>24.14</td>
<td>30.46</td>
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<tr>
<td>72</td>
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<td>0.40</td>
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<td>0.00</td>
<td>9.00</td>
<td>15.51</td>
<td>21.28</td>
<td>52.71</td>
</tr>
</tbody>
</table>

Table 4. Percentage of wound area remained in time interval of 96 hours on treatment with four different concentrations of EGCG

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<th>Percentage of wound area over time</th>
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<th>50</th>
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<th>250</th>
<th>500</th>
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<td>21.28</td>
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</tr>
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Multiple malignancies, including NSCLC, involve signalling molecules such as p53, EGFR, KRAS/MAPK, STAT3, NF-κB, and PI3K/AKT. These signaling cascades play a critical role in cancer cell growth and proliferation [34]. In vitro, EGCG has been found to decrease proliferation and induce apoptosis in several human cancer cell lines, including breast, melanoma, breast, lung, and colon. EGCG has also been demonstrated to induce apoptosis and limit metastasis in a range of cancer cell lines, including breast, prostate, liver, and lung cancer cells, by causing cell-cycle arrest or activating the mitogen-activated protein kinase cascade [35, 36]. The chemopreventive/antiproliferative activity of EGCG was tested in the T24 human bladder cancer cell line. EGCG treatment resulted in dosage- and time-dependent suppression of cell growth and viability, as well as triggered apoptosis [37]. According to the cancer genome atlas data, STAT1 is an oncogene in lung cancer, and EGCG significantly reduced STAT1 expression in A549 cells [38]. A study similar to ours, used the MTT assay to see how increasing the concentration of EGCG affected the proliferation of estrogen receptor-negative MDA-MB-231 human breast cancer cells over 24, 48, 72, and 96 hours. Treatment with EGCG (1-200 mg/ml) suppressed the proliferation of MDA-MB-231 breast cancer cells in a concentration-dependent manner, with an IC50 of 50 mg/ml at 48 hours. Cell growth was significantly inhibited in polyphenol treatment groups that received high concentrations (50 mg/ml) of EGCG [39]. In vitro studies showed that EGCG can decrease the development of lung cancer cells by modulating numerous signal transduction pathways [18]. EGCG exhibits antioxidant or pro-oxidant activities depending on dosage and exposure period. EGCG inhibits the progression of cell cycles and affects signaling pathways involved in cell growth and dif-
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Table 5. Rate of A549 cell migration on treatment with EGCG on varying concentrations at 96 hours study

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Rate of migration (μm/h)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
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<tr>
<td>24</td>
<td>39.75</td>
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<td>48</td>
<td>24.50</td>
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<td>72</td>
<td>17.21</td>
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<tr>
<td>96</td>
<td>12.98</td>
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Finally, the current study may provide a solid foundation for further research into the tumour suppressor EGCG. EGCG suppressed cell growth in A549 cells in a dose- and time-dependent manner.
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Conclusion

The protective effect of EGCG on A549 lung cancer cells is duration and dose dependent. This shows that EGCG may be useful in the prevention and treatment of lung cancer. EGCG performs well in the study and works as an inhibitor of a variety of pathways that promote cancer cell proliferation. The performance of EGCG and the other data reported in the study opens up new avenues for further investigation. Such research could serve as the foundation for the development of complex treatment techniques incorporating EGCG, a naturally occurring chemical with a variety of beneficial properties.

Limitations and future perspectives

There are some limitations to the current investigation. To begin, this study of EGCG was conducted only on A549 cells, and the method by which it suppresses A549 cell growth was not investigated due to laboratory conditions. Our next research will focus on the impact of EGCG on different lung cancer cell lines, as well as in-vitro studies on numerous signalling pathways that EGCG targets. The other drawback is that EGCG has low absorption in the human body, even at high doses [46]. Many studies have found that using nanoscale EGCG improves its bioavailability in the human body [47]. This will also be investigated in our future study.

Acknowledgements

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Disclosure of conflict of interest

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References

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