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Original Article

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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cells. 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 Imai 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 CO_2 incubator with a constant supply of 5% CO_2 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 1×10^4 cells per well. Following 24 hours of incubation, the culture was treated with different doses of EGCG

(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 × 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 (1×10^6 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 uM, 250 uM, 100 uM, and 50 uM). 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 (%) = $[(Area_{t0h} - Area_{t\Delta h})/Area_{t0h}] \times 100$

Where, $Area_{t0h}$ is the area of the wound at zero hours of compound treatment, and $Area_{t\Delta h}$ 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) = $(W_{t0h} - W_{t\Delta h})/t$

Where, W_0 is the width of the wound at 0 hour, $W_{t\Delta h}$ 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 lesser 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-

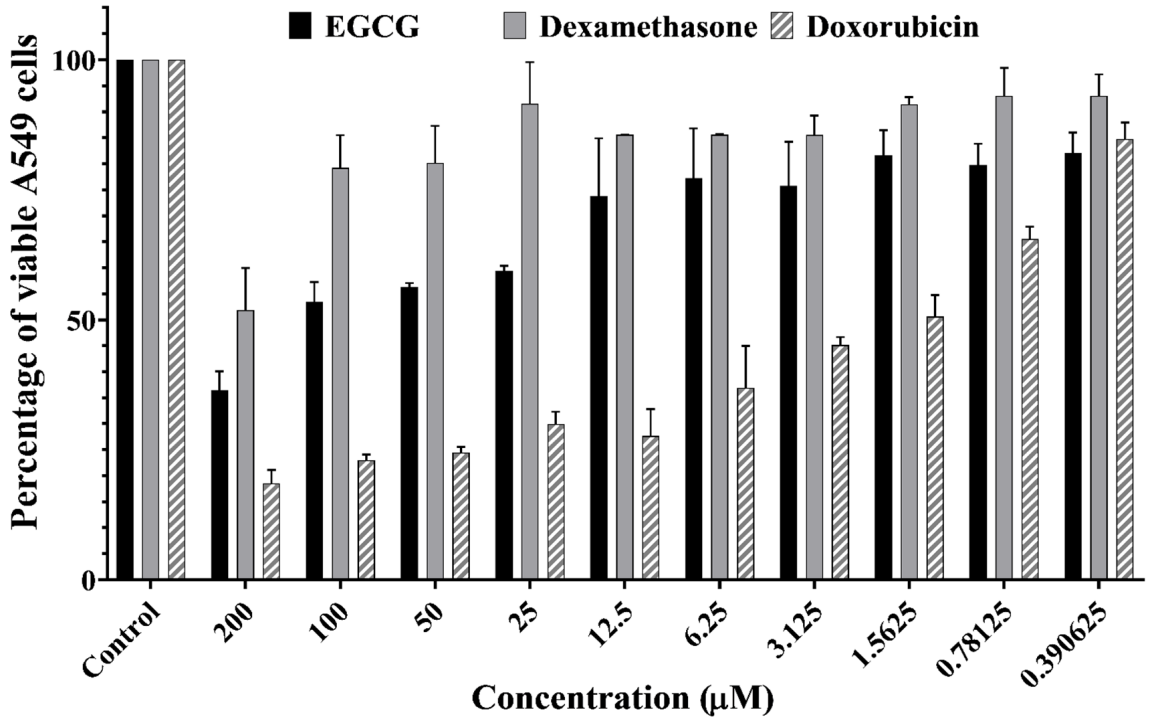

Figure 1. Group bar diagram of the viable A549 cells representing anti-proliferative activity on A549 cancer cells.

Table 1. IC50 values of anti-proliferative activity of test compounds on A549 lung adenocarcinoma cells

Test Compounds	IC50 Values (µM)
Epigallocatechin gallate	60.55 ± 1.0
Dexamethasone	227.9 ± 3.7
Doxorubicin	3.9 ± 0.2

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

Tukey's multiple comparisons test	Significant?	*P* Value
Dexa vs. EGCG	Yes	0.0016
Doxy vs. EGCG	Yes	0.0018

ent 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-

Table 3. Percentage of wound area remained in time interval of 96 hours on treatment with four different concentrations of EGCG

Time (hours)	Percentage of wound area over time				
	Control	EGCG (μM)			
		50	100	250	500
0	100.00	100.00	100.00	100.00	100.00
24	17.56	73.36	77.91	86.55	91.48
48	5.79	24.14	30.46	48.89	77.39
72	0.40	12.98	20.72	24.78	74.66
96	0.00	9.00	15.51	21.28	52.71

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

Time (hours)	Percentage of wound confluence over time				
	Control	EGCG (μM)			
		50	100	250	500
0	0.00	0.00	0.00	0.00	0.00
24	82.44	26.64	22.09	13.45	8.52
48	94.21	75.86	69.54	51.11	22.61
72	99.60	87.02	79.28	75.22	25.34
96	100.00	91.00	84.49	78.72	47.29

tality rate and a poor prognosis. Conventional treatments are usually ineffective in chronic and invasive lung tumours [30]. New therapeutic techniques, such as targeted therapy, have been identified as having the potential to enhance patient survival [31]. Plant-derived bioactive compounds have been shown to have anticancer activity as a targeted treatment agent [4].

Epigallocatechin-3-gallate (EGCG) is an active ingredient found in green tea, and its involvement in disease prevention as well as treatment has been shown. Its importance in disease management is due to its anti-inflammatory and antioxidant qualities. EGCG's anti-cancer properties have been demonstrated in a variety of cancers and are continuously being investigated. EGCG has been shown to have a chemopreventive impact by inhibiting carcinogenic processes including initiation, promotion, and development [18]. According to epidemiological and experimental research, EGCG has specific characteristics that may function as the foundation for considering them as key compounds in the synthesis of new anticancer drugs, as well as further investigation of their utility as pharmacologically active organic adjuvants to normal chemotherapy medications [32]. Furthermore, a lot of studies have reported the anti-cancer efficacy of EGCG. EGCG was helpful in reducing the likelihood of numerous cancer types, including stomach, prostate, and lung cancers by causing apoptosis, inhibiting metastasis, and angiogenesis [33].

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 leukemia, 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 (050 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-

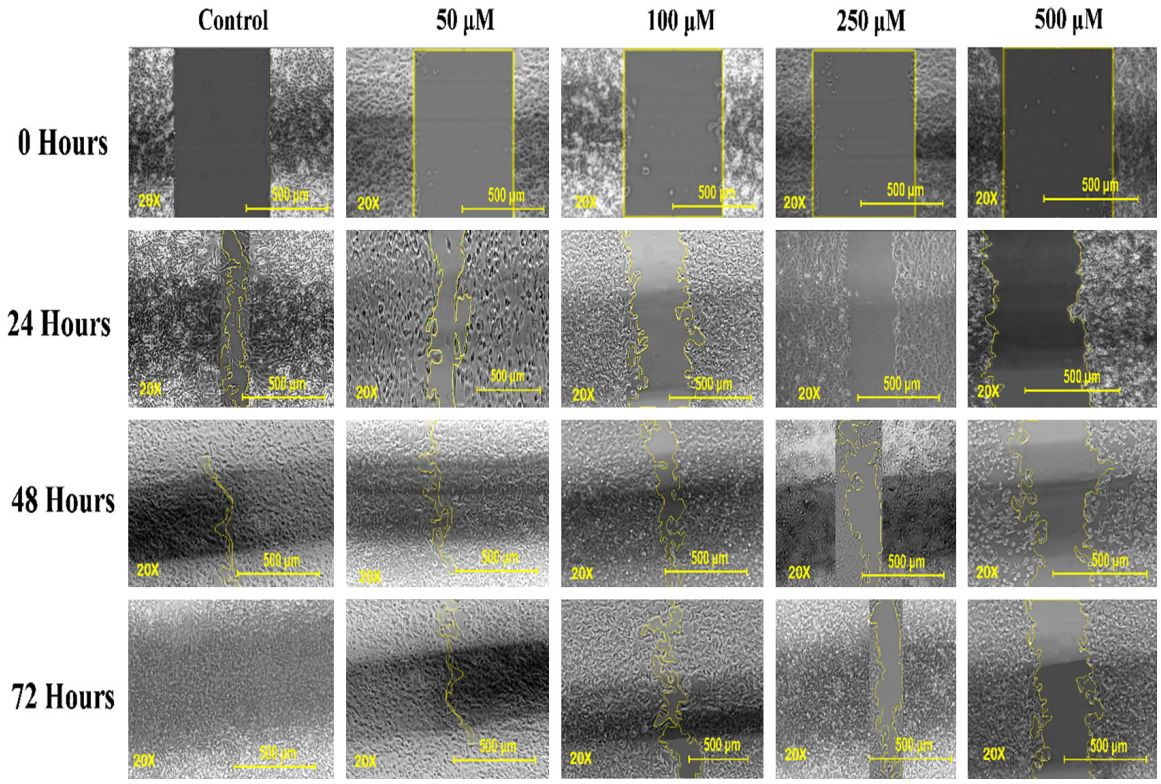

Figure 2. Wound Healing assay on A549 cells processed in imageJ software. Images represents the wound enclosure in 72 hours post treatment with EGCG compound with four concentrations 50, 100, 250, and 500 µM at 20X magnification.

Table 5. Rate of A549 cell migration on treatment with EGCG on varying concentrations at 96 hours study

Time (hours)	Rate of migration (µm/h)				
	Control	EGCG (µM)			
		50	100	250	500
0	0	0	0	0	0
24	39.75	8.92	8.04	2.67	1.92
48	24.50	8.75	5.63	5.71	5.17
72	17.21	9.08	6.06	6.25	4.96
96	12.98	7.90	7.66	6.17	3.96

ferentiation. EGCG also promotes apoptosis, affects many stages of metastasis, and inhibits VEGF transcription, which targets angiogenesis. In vivo, studies have revealed that oral treatment of EGCG reduces tumor growth and has antimetastatic and antiangiogenic properties in animal xenograft and allograft models [40]. In another study, EGCG dramatically lowered the expression levels of NF-κB and p-NF-κB in a dose-dependent manner [41]. NF-κB regulates genes that are downstream like Bcl-2, Bcl-xL, survivin, and COX-2, affecting cell proliferation, differentiation, apoptosis, invasion, and angiogenesis [42]. EGCG can reduce the growth and promote apoptosis of lung cancer cells, and this effect is linked to the suppression of the PI3K/Akt signaling pathway [43]. In a research, it was discovered that EGCG inhibited A549 cell growth in a dose-dependent manner which complements the results of our study [44]. The study conducted by Minnelli C et al., found that EGCG inhibited the proliferation of NSCLC cell line A-549 with a minimum inhibitory concentration (MIC) of 25 µM. EGCG exhibited a high MIC (100 µM) against normal human fibroblasts cells [45]. EGCG has been found to impact various signaling pathways in different studies, but the exact mechanism still needs to be investigated.

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.

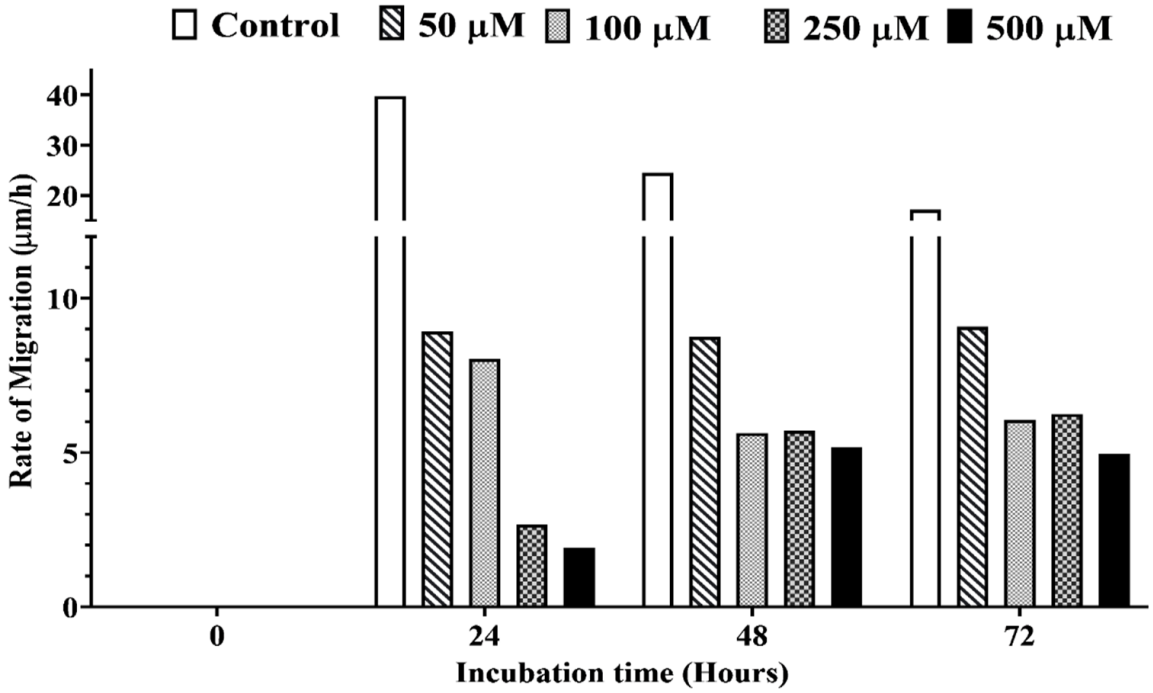

Figure 3. Rate of migration of A549 cells in scratch assay.

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.

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Disclosure of conflict of interest

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References

[1] Jones GS and Baldwin DR. Recent advances in the management of lung cancer. Clin Med (Lond) 2018; 18 Suppl 2: s41-s46.

[2] Rodriguez-Canales J, Parra-Cuentas E and Wistuba II. Diagnosis and molecular classification of lung cancer. Cancer Treat Res 2016; 170: 25-46.

[3] Schabath MB and Cote ML. Cancer progress and priorities: lung cancer. Cancer Epidemiol Biomarkers Prev 2019; 28: 1563-1579.

[4] Herbst RS, Morgensztern D and Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018; 553: 446-454.

[5] Alduais Y, Zhang H, Fan F, Chen J and Chen B. Non-small cell lung cancer (NSCLC): a review of risk factors, diagnosis, and treatment. Medicine (Baltimore) 2023; 102: e32899.

[6] Mithoowani H and Febbraro M. Non-small-cell lung cancer in 2022: a review for general practitioners in oncology. Curr Oncol 2022; 29: 1828-1839.

[7] Srivastava A, Amreddy N, Babu A, Panneerselvam J, Mehta M, Muralidharan R, Chen A, Zhao YD, Razaq M, Riedinger N, Kim H, Liu S, Wu S, Abdel-Mageed AB, Munshi A and Ramesh R. Nanosomes carrying doxorubicin exhibit potent anticancer activity against human lung cancer cells. Sci Rep 2016; 6: 38541.

[8] Dou XQ, Wang H, Zhang J, Wang F, Xu GL, Xu CC, Xu HH, Xiang SS, Fu J and Song HF. Aptamer-drug conjugate: targeted delivery of doxorubicin in a HER3 aptamer-functionalized liposomal delivery system reduces cardiotoxicity. Int J Nanomedicine 2018; 13: 763-776.

[9] Wang LJ, Li J, Hao FR, Yuan Y, Li JY, Lu W and Zhou TY. Dexamethasone suppresses the growth of human non-small cell lung cancer via inducing estrogen sulfotransferase and inactivating estrogen. Acta Pharmacol Sin 2016; 37: 845-856.

[10] Mohammadi L, Mosayyebi B, Imani M, Zarghami N, Alizadeh E and Rahmati M. Correlation between dexamethasone and miRNAs in the regulation of apoptosis, drug-resistance, and metastasis of cancer cell. Curr Mol Med 2021; 21: 392-401.

[11] Chung YJ, Lee JI, Chong S, Seok JW, Park SJ, Jang HW, Kim SW and Chung JH. Anti-proliferative effect and action mechanism of dexamethasone in human medullary thyroid cancer cell line. Endocr Res 2011; 36: 149-157.

[12] Ayyildiz A, Koc H, Turkekul K and Erdogan S. Co-administration of apigenin with doxorubicin enhances anti-migration and antiproliferative effects via PI3K/PTEN/AKT pathway in prostate cancer cells. Exp Oncol 2021; 43: 125-134.

[13] Momchilova A, Pankov R, Staneva G, Pankov S, Krastev P, Vassileva E, Hazarosova R, Krastev N, Robev B, Nikolova B and Pinkas A. Resveratrol affects sphingolipid metabolism in A549 lung adenocarcinoma cells. Int J Mol Sci 2022; 23: 10870.

[14] Maiuolo J, Gliozzi M, Carresi C, Musolino V, Oppedisano F, Scarano F, Nucera S, Scicchitano M, Bosco F, Macri R, Ruga S, Cardamone A, Coppoletta A, Mollace A, Cognetti F and Mollace V. Nutraceuticals and cancer: potential for natural polyphenols. Nutrients 2021; 13: 3834.

[15] Zhou Y, Zheng J, Li Y, Xu DP, Li S, Chen YM and Li HB. Natural polyphenols for prevention and treatment of cancer. Nutrients 2016; 8: 515.

[16] Hayakawa S, Ohishi T, Miyoshi N, Oishi Y, Nakamura Y and Isemura M. Anti-cancer effects of green tea epigallocatchin-3-gallate and coffee chlorogenic acid. Molecules 2020; 25: 4553.

[17] Alam M, Ali S, Ashraf GM, Bilgrami AL, Yadav DK and Hassan MI. Epigallocatechin 3-gallate: from green tea to cancer therapeutics. Food Chem 2022; 379: 132135.

[18] Almatroodi SA, Almatroudi A, Khan AA, Alhumaydhi FA, Alsahli MA and Rahmani AH. Potential therapeutic targets of epigallocatechin gallate (EGCG), the most abundant catechin in green tea, and its role in the therapy of various types of cancer. Molecules 2020; 25: 3146.

[19] Liang YC, Lin-Shiau SY, Chen CF and Lin JK. Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (-)-epigallocatechin-3-gallate. J Cell Biochem 1999; 75: 1-12.

[20] Singh BN, Shankar S and Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol 2011; 82: 1807-1821.

[21] Kurahashi N, Sasazuki S, Iwasaki M, Inoue M and Tsugane S; JPHC Study Group. Green tea consumption and prostate cancer risk in Japanese men: a prospective study. Am J Epidemiol 2008; 167: 71-77.

[22] Shimizu M, Fukutomi Y, Ninomiya M, Nagura K, Kato T, Araki H, Suganuma M, Fujiki H and Moriwaki H. Green tea extracts for the prevention of metachronous colorectal adenomas: a pilot study. Cancer Epidemiol Biomarkers Prev 2008; 17: 3020-3025.

[23] Fujiki H, Watanabe T, Sueoka E, Rawangkan A and Suganuma M. Cancer prevention with green tea and its principal constituent, EGCG: from early investigations to current focus on human cancer stem cells. Mol Cells 2018; 41: 73-82.

[24] Freitas JT, Jozic I and Bedogni B. Wound healing assay for melanoma cell migration. Methods Mol Biol 2021; 2265: 65-71.
[25] Martinotti S and Ranzato E. Scratch wound healing assay. Methods Mol Biol 2020; 2109: 225-229.
[26] Bossio S, Perri A, Malivindi R, Giordano F, Rago V, Mirabelli M, Salatino A, Brunetti A, Greco EA and Aversa A. Oleuropein counteracts both the proliferation and migration of intra- and extragonadal seminoma cells. Nutrients 2022; 14: 2323.
[27] Deng L, Zou J, Su Y, Wang M and Zhao L. Resveratrol inhibits TGF-β1-induced EMT in gastric cancer cells through Hippo-YAP signaling pathway. Clin Transl Oncol 2022; 24: 2210-2221.
[28] Luo KW, Xia J, Cheng BH, Gao HC, Fu LW and Luo XL. Tea polyphenol EGCG inhibited colorectal-cancer-cell proliferation and migration via downregulation of STAT3. Gastroenterol Rep (Oxf) 2020; 9: 59-70.
[29] Filippi A, Picot T, Aanei CM, Nagy P, Szöllősi J, Campos L, Ganea C and Mocanu MM. Epigallocatechin-3-O-gallate alleviates the malignant phenotype in A-431 epidermoid and SK-BR-3 breast cancer cell lines. Int J Food Sci Nutr 2018; 69: 584-597.
[30] Lemjabbar-Alaoui H, Hassan OU, Yang YW and Buchanan P. Lung cancer: biology and treatment options. Biochim Biophys Acta 2015; 1856: 189-210.
[31] Fraga CG, Croft KD, Kennedy DO and Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food Funct 2019; 10: 514-528.
[32] Farhan M. Green tea catechins: nature's way of preventing and treating cancer. Int J Mol Sci 2022; 23: 10713.
[33] Lin JK and Liang YC. Cancer chemoprevention by tea polyphenols. Proc Natl Sci Counc Repub China B 2000; 24: 1-13.
[34] Hasan GM, Hassan MI, Sohal SS, Shamsi A and Alam M. Therapeutic targeting of regulated signaling pathways of non-small cell lung carcinoma. ACS Omega 2023; 8: 26685-26698.
[35] Khan N, Afaq F, Saleem M, Ahmad N and Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. Cancer Res 2006; 66: 2500-2505.
[36] Aggarwal BB and Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 2006; 71: 1397-1421.
[37] Qin J, Xie LP, Zheng XY, Wang YB, Bai Y, Shen HF, Li LC and Dahiya R. A component of green tea, (-)-epigallocatechin-3-gallate, promotes apoptosis in T24 human bladder cancer cells via modulation of the PI3K/Akt pathway and Bcl-2 family proteins. Biochem Biophys Res Commun 2007; 354: 852-857.
[38] Li F, Hao S, Gao J and Jiang P. EGCG alleviates obesity-exacerbated lung cancer progression by STAT1/SLC7A11 pathway and gut microbiota. J Nutr Biochem 2023; 120: 109416.
[39] Thangapazham RL, Singh AK, Sharma A, Warren J, Gaddipati JP and Maheshwari RK. Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells in vitro and in vivo. Cancer Lett 2007; 245: 232-241.
[40] Romano A and Martel F. The role of EGCG in breast cancer prevention and therapy. Mini Rev Med Chem 2021; 21: 883-898.
[41] Zhang L, Xie J, Gan R, Wu Z, Luo H, Chen X, Lu Y, Wu L and Zheng D. Synergistic inhibition of lung cancer cells by EGCG and NF-κB inhibitor BAY11-7082. J Cancer 2019; 10: 6543-6556.
[42] Bensassi F, El Golli-Bennour E, Abid-Essefi S, Bouaziz C, Hajlaoui MR and Bacha H. Pathway of deoxynivalenol-induced apoptosis in human colon carcinoma cells. Toxicology 2009; 264: 104-109.
[43] Gu JJ, Sun P, Qiao KS, Chen P and Li Q. Study of EGCG induced apoptosis in lung cancer cells by inhibiting PI3K/Akt signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 4557-4563.
[44] Sakamoto Y, Terashita N, Muraguchi T, Fukusato T and Kubota S. Effects of epigallocatechin-3-gallate (EGCG) on A549 lung cancer tumor growth and angiogenesis. Biosci Biotechnol Biochem 2013; 77: 1799-1803.
[45] Minnelli C, Cianfruglia L, Laudadio E, Mobbili G, Galeazzi R and Armeni T. Effect of epigallocatechin-3-gallate on EGFR signaling and migration in non-small cell lung cancer. Int J Mol Sci 2021; 22: 11833.
[46] Oritani Y, Setoguchi Y, Ito R, Maruki-Uchida H, Ichiyanagi T and Ito T. Comparison of (-)-epigallocatechin-3-O-gallate (EGCG) and O-methyl EGCG bioavailability in rats. Biol Pharm Bull 2013; 36: 1577-1582.
[47] Karimi-Shahri M, Alalikhan A, Hashemian P, Hashemzadeh A and Javid H. The applications of epigallocatechin gallate (EGCG)-nanogold conjugate in cancer therapy. Nanotechnology 2023; 34.