J HerbMed Pharmacol. 2015; 4(2): 65-68.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Effect of Epigallocatechin-3-gallate (EGCG) on cell proliferation inhibition and apoptosis induction in lymphoblastic leukemia cell line

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 13 December 2014
Accepted: 7 February 2015

Keywords:
EGCG
Proliferation
Jurkat cell line
Acute lymphoblastic leukemia
Apoptosis

ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) is one of the malignant proliferations of lymphoid cells in the early stages of differentiation and accounts for ¾ of all cases of childhood leukemia. Available treatment cannot completely treat this disease. Epigallocatechin-3-gallate (EGCG) is a polyphenolic compounds in the green tea that has demonstrated to have anticancer and antimitotic properties. The purpose of the present study was the evaluation of the effect of EGCG on the proliferation inhibition and apoptosis induction in a lymphoblastic leukemia cell line.

Methods: Jurkat cell line was cultured in standard condition and in different concentrations of EGCG (0-100 micromolar) for 24, 48 and 72 hours. Cell viability was measured by MTS assay. Apoptosis induction was assessed by annexin V-FITC and flow cytometry analysis.

Results: The MTS assay revealed that EGCG has decreased cell viability with a time and dose dependent manner. The level of cell apoptosis in all used concentrations of EGCG (50, 70 and 100 µm) was higher than control group (71%, 40% and 31% respectively vs. 8%) and reached to significant level at 100 µm concentration.

Conclusion: The study indicated that EGCG is effective on proliferation inhibition and apoptotic induction in Jurkat lymphoblastic cell line. Therefore, the study of the mechanism of apoptosis induction could be a step of progress toward target therapy which might be considered in the future studies.

Implication for health policy/practice/research/medical education:
EGCG has anti-cancer properties against different cell lines and this effect seems to be through apoptosis induction in lymphoblastic leukemia. These results can be a step toward targeted combination or alternative chemotherapy in cancer.

Please cite this paper as: Ghasemi-Pirbaluti M, Pourgheysari B, Shirzad H, Motaghi E, Askarian Dehkordi N, Surani Z, *et al.* Effect of Epigallocatechin-3-gallate (EGCG) on cell proliferation inhibition and apoptosis induction in lymphoblastic leukemia cell line. J HerbMed Pharmacol 2015; 4(2): 65-68.

Introduction

Cancer is the second or third cause of death in developing countries. It has been estimated that 15 million people will die from cancer in 2020 (1). Hematological malignancies originate from hematopoietic cells in the bone marrow or lymphoid organs (2).

Acute lymphoblastic leukemia (ALL) is the most common form of leukemia in the children with the incidence of 40 per million. Thirty percent of children with cancer under 15 years old suffer from ALL (3,4).

Although new treatments in last decade improved the quality of life, cytotoxic drugs cannot completely cure the cancer. On the other hand, their side effects may kill some of the patients. Plant derived compounds can help in cancer treatment by growth inhibition (5).

Recently, scientists are trying to find out natural

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compounds and their derivatives to treat different diseases including cancer. Many studies have shown that plant derivatives are apoptotic inducer that can inhibit cancer (6).

Above 60% of available anticancer drugs have been derived from plants, plants organs or have microbial origin (7). Epigallocatechin-3-gallate (EGCG) is a polyphenolic compound with anticancer properties against many cell lines including (8) B cell lymphoblastic leukemia (9). EGCG has been found to potentiate the inhibitory effect of Ziziphus extracts on hepatocarcinoma cells (10).

Apoptotic pathway is an important target in effective treatment of cancer (11). Previous studies indicated that EGCG could inhibit splenic B lymphocyte (12) and mice lymphocyte proliferation (13). On the other report, this compound can induce neopterine (an immunological marker) in mononuclear cell in blood (14).

To the best of our knowledge, there is no study describing the effect of EGCG on lymphoblastic leukemia cell line. Evaluation of the effect of EGCG on lymphoblastic cells and its effective doses can help to identify of the anticancer properties of polyphenolic compounds.

This study was aimed to investigate the effects of physiological and pharmacological concentrations of EGCG on Jurkat lymphoblastic leukemia cell line.

Materials and Methods

A lymphoblastic cell line (Jurkat E6.1) was grown in RPMI-1640 culture medium supplemented with 0.3 mg/ml glutamine, 10% fetal calf serum, 100 IU penicillin and 100 µg streptomycin, and kept at 37°C in a humidified atmosphere containing 5% CO2. Cells in the exponential phase were used for all experiments. Cell count was performed by hemocytometer and tripan blue dye.

Analysis of cell viability (MTS assay)

MTS/PMS was used to assay viability and proliferation of the cell line according to Thabrew method (15). Briefly, cells at 1×10^4 cell/ml were cultured in 96-well flat-bottomed tissue culture plate. The cells were treated with EGCG (0-100 micromolar) for 24, 48 and 72 hours in 37°C, 5% CO2 and humidified atmosphere. At the end of the experiment, MTS/PMS solution was added to each well and cells were incubated for 2 hours at 37°C in a dark place. The absorbance at 490-620 nm was assessed using ELISA reader (stat fax-2100 awareness). Each independent experiment was repeated at least tree times and the concentration required for a 50% inhibition of growth (IC50) was determined.

Annexin V/PI assay

Apoptosis was examined by flow-Cytometry with the FITC Annexin V Apoptosis Detection Kit (BD Pharmingen). Briefly, the cells were treated with different concentrations of EGCG. Then the cells were harvested and washed in PBS twice. 10^6 cells were re-suspended in 1 ml 1 X binding buffer. 10^5 cells were incubated with 5 µl of FITC-labeled annexin V and 1 µl of 100 µg/ml PI (propidium iodide) at room temperature in dark place for 20 minutes, then 400 µl of 1 X binding buffer were added. The tube mixed gently, and kept on ice and analyzed by flow-Cytometry equipment (PARTEC).

Statistical analysis

All data are presented as mean±SEM. Calculations and statistical tests were performed using SPSS (SPSS) and GraphPad Prism (v5.01, USA). Comparisons of data were made using Kruskal–Wallis test. A $P\leq0.05$ was considered significant. All experiments were repeated at least 3 times.

Results

EGCG significantly decreased cell proliferation in Jurkat line after 24, 48 and 72 hours incubation. The ratios of viable cells to control ones were 22%, 51% and 60% in 50, 70 and 100 micromolar concentrations, respectively after 48 hours. The ratios of viable cells to control ones were 16%, 38% and 52% in 50, 70 and 100 micromolar concentrations, respectively after 72 hours (Figure 1).

Effect of different concentration of EGCG on apoptosis

EGCG was effective in different concentrations on the cell line and the drug induced apoptosis successfully, shown by MTS/PMS assay. Apoptosis was increased significantly after treatment with different concentrations of EGCG compared to control group.

Figure 2 shows the percent of Jurkat cell apoptosis in the presence of 70 micromolar concentration of ECGC. 50, 70 and 100 micromolar concentrations of EGCG induced apoptosis in 31%, 40% and 71% of the cells, respectively (Figure 3).

Discussion

Apoptosis is the process of cell self destruction (PCD) that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology), the fragmentation of nuclear DNA and death. Decrease in apoptosis rate can lead frequently to genetic change and cancer (16).

The study investigated the anti-proliferative effects of

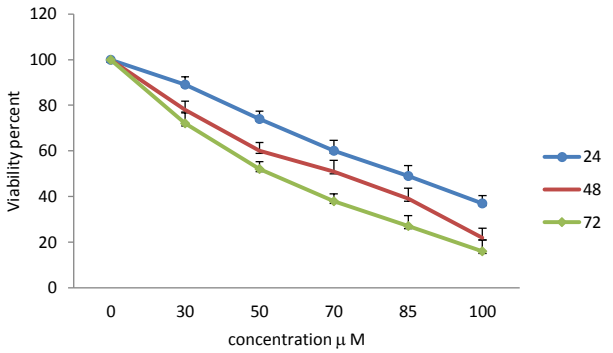

Figure 1. Effect of different concentrations of EGCG on viability of Jurkat cell line after 24, 48, and 72 hours.

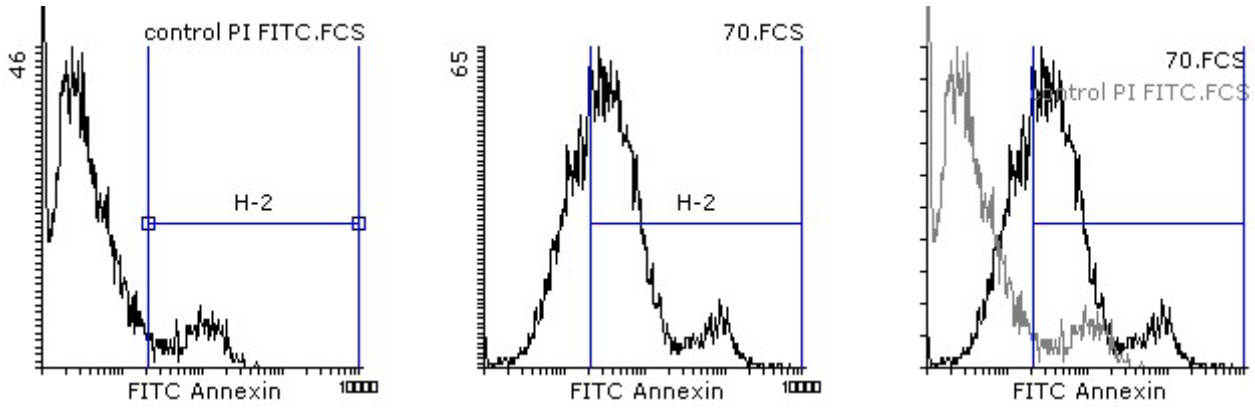

Figure 2. Flow cytometric analysis of apoptosis of Jurkat cells treated with EGCG (black curve) compared to control cells (gray curve). Percents of apoptosis were 8% and 40%, respectively in control and EGCG groups.

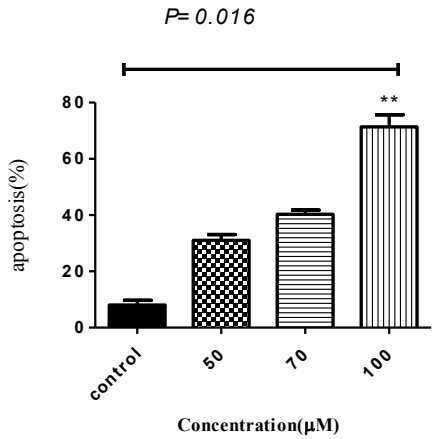

Figure 3. The effect of different concentrations of EGCG on Jurkat cell apoptosis after 48 hours incubation compared to control group (**P<0.01 show the significant difference).

EGCG on Jurkat cell line.

According to the results of MTS/PMS assay, different concentrations of EGCG decreased viability of Jurkat cells, dose and time dependently.

Sturgeon et al showed that green tea can notably decrease incidence and improve prognosis of some cancers including intestine, lung, pancreatic, and prostate cancers (17). Previous studies have shown the antioxidant properties of green tea against oxidative induced DNA damage or lipid and protein oxidation (18).

Annabi et al. reported that EGCG inhibited expression of MMP-9 gene, which had a significant role in cancer cell growth and metastasis, in HL-60 myeloid human leukemia cells (19).

Smith et al found that the EGCG inhibits DNA replication in different leukemia cell lines including HL-60. EGCG is the most potent in S phase compared to other cellular phase (20).

Our study indicated that EGCG inhibited successfully the proliferation of Jurkat cells and induced cell apoptosis at different concentrations. Apoptosis in lymphoblastic Jurkat cells is a suitable model for mechanistic investigation of the EGCG on cell growth. EGCG has inhibitory effect in MCF-7 (breast cancers cells) and HL60 promyelocytic leukemia cells (21). The results of our study are in agreement with the other studies results. In our study, we observed that EGCG could induce dose dependently apoptosis in Jurkat cells, similar to previous studies in other cancer cell lines.

Conclusion

The Results of the study showed that EGCG is effective in proliferation and apoptosis induction in Jurkat cell line. The drug can be considered as a potential for lymphoblastic leukemia chemotherapy. The study of the mechanism of apoptosis in lymphoblastic cell lines besides in vivo studies in experimental models can be a progress toward targeted chemotherapy in cancer.

Acknowledgments

This paper was derived from MSc thesis of the first author, which was supported by the Medical Plants Research Center of Shahrekord University of Medical Sciences. The authors also wish to thank deputy of Research, Shahrekord University of Medical Sciences for financial support.

Authors' contributions

MG performed the experimental work and helped the writing, BP led the design and writing the project, HS helped with the design , EM, NA, ZS, MS and AP helped in experimental work and analysis, PB led the flowcytometry analysis.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

This research was financially supported by Shahrekord University of Medical Sciences, Shahrekord, Iran.

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