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The effect of gallic acid on Jurkat cell line

Zahra Sourani[1], Batoul Pourgheysari[2*], Mahmoud Rafieian-Kopaei[2], Hedayatollah Shirzad[3], Moien Shirzad[4]

[1]Msc Student in Immunology, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[3]Cellular & Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4]Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) is the most prevalent leukemia in children. Fruits and plants have a wide range of biological functions including anti-proliferative effect. Gallic acid (GA), is a polyhydroxyphenolic compound that is widely distributed in the natural plants. The aim of the present study was the evaluation of the effect of GA on proliferation inhibition of Jurkat cells, the lymphoblastic leukemia cell line.
Methods: Jurkat cell line was cultured in blood cells culture media in a standard conditions with different concentrations of GA (0-100 µm) for 24, 48 and 72 hours. The effect of GA on cell viability was measured using MTS assay.
Results: Decline of cell viability to less than 50% was observed at 60, 50 and 30 µm concentrations after 24, 48 and 72 hours incubation time, respectively.
Conclusion: The results demonstrated that the polyphenolic compound, GA with antioxidant capability is effective in proliferation inhibition in Jurkat lymphoblastic leukemia cell line with a time and dose dependent manner. Therefore, GA may be a potential compound for cancer prevention and treatment.

Implication for health policy/practice/research/medical education:
Gallic Acid (GA) has anti-cancer property 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.
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Introduction

Acute lymphoblastic leukemia (ALL) is a malignancy of immature lymphoid cells. The over production of malignant cells in the bone marrow inhibits the production of other blood cells. ALL is the most prevalent malignancy in children, representing 25%-30% of all childhood malignancies (1). Many plants contain components against cancer cells that can induce their effects in different stages of growth of cancer cells (2). A promising strategy for cancer prevention is introducing drugs that block the development of cancer (3). Food plays an important role in preventing the onset and progression of cancer. Nowadays, medicinal plants have attracted the attention of many cancer patients around the world. The scientists have focused on the biological and therapeutic properties of these products (4). Induction of apoptosis in cells is one of the targets of anti-cancer therapy (5). Gallic acid (GA) is a polyhydroxyphenolic compound that is widely distributed in the natural plants, fruits and food and has a wide range of biological functions (6). Many foods such as gallnuts, sumac, grape, green tea, oak bark, strawberry, lemon, banana, pineapple, witch hazel, and apple peel are known to be rich in GA (7). GA and its analogs have been reported to have many biological activities, including antioxidant property (8), anti-tumor (7), anti-mutagenic, and anti-carcinogenic properties (9). However, the major interest in GA and its analogs is related to its antitumor activity. It has been shown that propyl, lauryl, methyl gallate induce apoptosis and inhibit proliferation in tumor cell lines (10). GA has shown no cytotoxicity against normal fibroblast and endothelial cells (11). Some studies have shown that GA causes DNA fragmentation (10) and is also responsible for suppression of tumor angiogenesis, leading to inhibition of tumor metastasis (12). In fact, anti-cancer activity of GA has been reported in various cancer cells, such as prostate (13), lung, gastric, colon, breast, cervical and

***Corresponding author:** Batoul Pourgheysari, Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Email: Bat238@yahoo.com

esophageal cancers (14). As there was no report of the effect of GA on lymphoblastic leukemia cell line, we aimed to evaluate the effect of GA on proliferation inhibition on this cell line.

Materials and methods

Cell culture

Lymphoblastic leukemia cell line (Jurkat cell) was cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100IU/mL penicillin, 0.1 mg/mL streptomycin (PS) and 0.3 mg/ml L-glutamine. The cells were incubated at 37°C in a humidified incubator with 5% carbon dioxide. The cells were maintained at the concentration of 2×10^5/ml and then transferred to 96 and/or 6-well plates for experiments. To determine the viable cells, the culture was harvested and the cells were counted by the trypan blue staining and standard hemocytometer.

Cell culture and treatment

Jurkat cells were cultured in RPMI-1640 medium containing 10% FBS. The stock solution of GA was dissolved in RPMI-1640, and different concentrations (uM) were prepared in the RPMI-1640 medium.

Assessment of cell viability by MTS assay

This chromogenic assay involves the biological reduction by viable cells of the tetrazolium compound 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (or MTS).

MTS assay reagent is composed of MTS and the electron coupling agent phenazine methosulfate (PMS). The formazan product of MTS reduction is soluble in tissue culture medium. This reaction only takes place when mitochondrial reductase enzymes are active, and therefore the conversion can be directly related to the viability of cells in culture. The MTS reagent alone results in very low background absorbance values in the absence of cells.

Jurkat cells were seeded in above complete media at the concentration of 10×10^3 cells per well in 96-well plates and allowed to grow overnight. The cells were treated with different concentrations (0-100 µM) of GA. After incubation for 48 hours, 20 µL of MTS (5 mg/mL in phosphate buffered saline) was added to each well and incubated for 3 hours, in the dark. The absorbance was measured at 490-620 nm by an Elisa reader (stat fax-2100 awarenes).

Statistical analysis

Data were analyzed by SPSS using Kruskal-Wallis test and are presented as the means ± standard deviation from at least three independent experiments.

Results

Decrease cell viability by gallic acid

Jurkat cells were incubated with various concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µM) of GA for 24, 48 and 72 hours. Decrease of cell viability was observed after incubation time in treated cells in a time–dependent manner (Figure 1). The MTS assay was done to investigate the cell viability. The data obtained from the absorption at 490 nm was converted to a percentage. The results showed a dose- and time-dependent behavior (Figure 2). Cell viability was about 93%, 87%, 65%, at a concentration of 20µM after 24, 48 and 72 hours, respectively, however viability declined to 13%, 8% and 5% at a concentration of 100µM after above incubation periods. IC_{50} (inhibition concentration) was about 60µM in 24 hours, about 50µM in 48 hours and about 30µM in 72 hours (Figures 2 and 3).

Discussion

Growth inhibition and antiproliferative effect of GA have been shown in some tumor cell lines (15). The results of our study show that GA in a dose and time dependent manner decreased cell proliferation in Jurkat cell line. The result showed IC_{50} about 60µM in 24 hours, about 50µM in 48 hours and about 30µM in 72 hours. The concentrations of drug had significant difference in cell viability in the MTS assay. GA inhibitory effect on cell proliferation has been studied in several cancer cell lines in various studies, which are in agreement with our findings. You and Park (16) have shown the effect of GA in 2 types of lung cancer

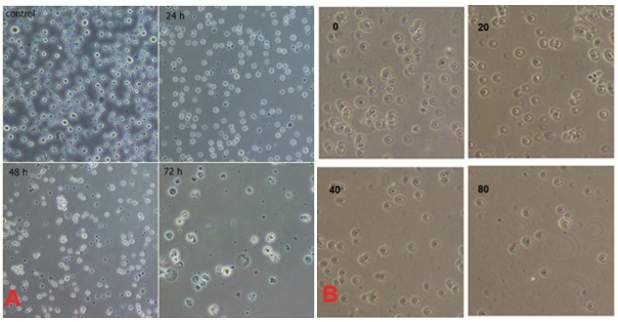

Figure 1. A: Decrease of cell viability in the presence of GA after 24, 48 and 72 hours (time-dependent). B: Decrease of cell viability in Jurkat cells incubated with various concentrations (0, 20, 40, 80) (dose-dependent) (Magnification 40x).

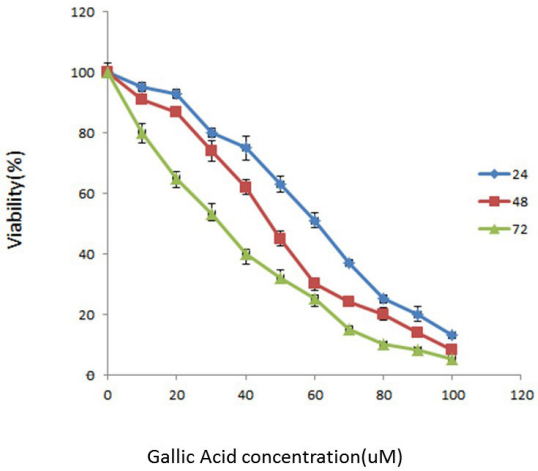

Figure 2. Jurkat cell viability by MTS assay. Cell viability was determined by measuring the mitochondrial metabolism of MTS after 24, 48 and 72 hours.

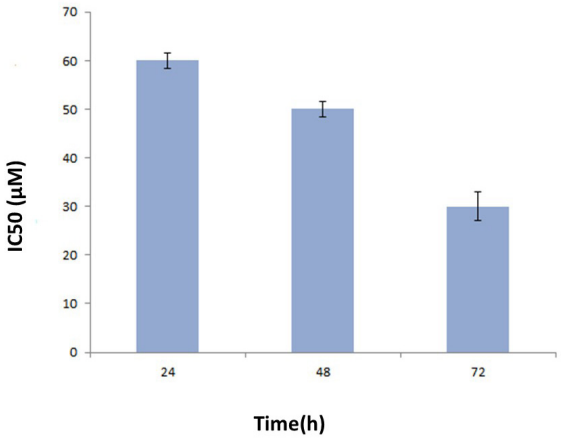

Figure 3. Comparison of the IC_{50} (50% inhibition concentration) of gallic acid treated cells. Data represent the mean ± SD. The effect of treatment duration is clear from the graph.

cells (A549 and Calu-6). The inhibition of the growth of lung cancer cells and induction of cell death were related to GSH (glutathione) depletion as well as reactive oxygen species (ROS) level changes. The induction of apoptosis has been shown to be related to ROS in Larry et al study in prostate cancer cells. In this study, 80 µg/ml GA has the maximum inhibitory effect in 24 hours and caused activation of apoptosis pathway (17). Chia et al (18) studied the anticancer effects of GA in oral cancer cell lines including UM1, UM2, SCC-4 and SEC-9. They showed that the required concentration of GA for the induction of apoptosis was different in various cell lines. Lu et al (12) have studied GA-induced cell death and the anti-proliferation, anti-invasion, and angiogenesis in human glioma cells. They showed that the inhibition of cell viability by GA was dose dependent. Madlener et al (19) studied the effect of GA in promyelocytic leukemia cells (HL-60) They showed that apoptosis induced by GA is dose dependent. Other studies have demonstrated the effect of GA on gastric cancer cells (20). You et al (7) studied the effect of GA in Hela cervical cancer cells. They showed that GA was able to inhibit the growth of Hela cells and to induce apoptosis. GA-induced Hela cell death was also accompanied by ROS increase and GSH depletion. The effect of GA on Leukemia cells (K562) and L1210 (15,21) was also in consistent with our data. Our study showed that GA could inhibit cell proliferation in lymphoblastic leukemia cell line which was consistent with the studies of other cell lines.

Conclusion

It seems from the study that decrease of Jurkat cell numbers in the presence of GA is dependent to the dose- and time which is a favorable effect of anti-cancer treatments. GA may help in cancer combination- chemotherapy but it needs to be investigated in other lymphoblastic cell lines, ALL lymphoblasts and then in vivo experimental models.

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Authors' contributions

ZS performed the experimental work and helped the writing; BP led the design and writing the project; HS and MR helped with the design; MS helped in experimental design and 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.

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