The effect of gallic acid on Jurkat cell line

Zahra Sourani1, Batoul Pourgheysari2*, Mahmoud Rafieian-Kopaei3, Hedayatollah Shirzad4, Moien Shirzad4

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

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.

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), 100 IU/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 $2 \times 10^5$/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 (µM) 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 $10 \times 10^3$ 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
Effect of gallic acid on Jurkat cell line

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.

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 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.

Acknowledgements
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
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.

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

References


