

A Novel Combination of Pterostilbene and Dexamethasone Enhances Anti-proliferation and Apoptosis Induction Properties in Lymphoblastic Leukemia Cell Line

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Abstract

Background: T-cell acute lymphoblastic leukemia is an aggressive hematologic malignancy that results from the transformation of T-cell progenitors. Despite the significant advances in the current treatments, the side-effects of conventional chemotherapy regimens are still a major concern. Pterostilbene (PT) is a natural compound reported to have antitumor effects. This study aimed to investigate the effect of PT combined with dexamethasone on the proliferation inhibition and apoptosis stimulation in a lymphoblastic leukemia cell line.

Methods: In this experimental study, we cultured Jurkat cell line in RPMI1640 culture medium under standard conditions. We incubated the cells with different concentrations of PT and dexamethasone, separately or in combination for 48 h. MTS assay investigated the cell viability. We assessed apoptosis induction by annexin V-FITC/PI and flow cytometry analysis.

Results: PT and dexamethasone reduced the viability of the cells with inhibition concentrations of 60.97 ± 3.36 and 451.1 ± 10.1 μM , respectively, in 48 h. None of the concentrations of dexamethasone, employed alone, significantly reduced the cell viability. The combination of 450 μM dexamethasone with 60 μM PT induced apoptosis in more than 70% of the cells with a significant difference compared to control.

Conclusion: PT increased the antiproliferative and apoptosis-inducing activity of dexamethasone in Jurkat cell line. This combination drug strategy can be a novel approach for a more powerful anticancer therapy.

Keywords: Apoptosis, Jurkat cell line, T-ALL, Dexamethasone, Pterostilbene

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Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignancy affecting the lymphoblastic lineage. Depending on the type of lymphoblastic lineage involved, ALL can be subdivided into T-cell ALL (T-ALL) and B-cell ALL (B-ALL).¹ T-ALL accounts for approximately 20% of adult and 10-15% of childhood ALL cases. Malignant transformation of T-cells may occur at any stage of maturation, leading to the marked heterogeneity of this leukemia.² This aggressive malignancy typically displays a very active proliferative level, a high tumor burden, numerous large thymic masses, and pleural effusions. Despite the significant improvement in the current treatments, T-ALL still remains a leading cause of cancer-related mortality in pediatrics.³ Prognosis in the elderly is still very poor.¹ Therefore, novel therapeutic approaches are still needed regarding patients unresponsive to conventional chemotherapy.

A favorable attitude for cancer prevention is introducing drugs that block the expansion of cancer. Several extracts of medicinal plants were applied to overcome multidrug resistance.⁴ Plant-derived components have been known to exert some effects on blood cells and factors.^{5, 6} Natural polyphenols affect various stages of cell cycle and proliferation.⁷

Resveratrol, the most investigated polyphenol, is a promising chemoprotective agent for cancer treatment. It is considered as an effective antitumor agent owing to its low toxicity and ability to regulate complex cancer-associated molecular

pathways.⁸

Pterostilbene (PT) (trans-3, 5-dimethoxy-4-hydroxystilbene) is a natural dimethylated analog of resveratrol and an antitumor compound derived from grapes and other foods.⁹ Similar to resveratrol, PT induces apoptosis and has antioxidant, anticancer, anti inflammation, and antiproliferation properties.¹⁰ PT has more oral uptake and bioavailability compared with resveratrol. In previous reports, PT induced cancer cell apoptosis in breast, liver, ovarian, and lung malignancies.¹¹⁻¹⁴

At the beginning of treatment with ALL, dexamethasone was increasingly used in the intensification phase of the disease.¹⁵ In this study, for the first time, we examined the effect of PT in combination with dexamethasone on the cell proliferation and apoptosis induction in T-lymphoblastic cell line.

Materials and Methods

In the present experimental study, we investigated the effect of PT combined with dexamethasone on the proliferation inhibition and apoptosis stimulation in a Jurkat cell line.

Cell culture

The National Cell Bank of Iran (Pasteur Institute of Iran, Tehran) provided the human T-cell leukemia cell line (Jurkat). Cells were grown in RPMI1640 culture medium supplemented with 10% fetal calf serum, 0.3 mg/ml glutamine, 100 IU penicillin, and 100 µg/ml streptomycin; they were then incubated in a humidified atmosphere

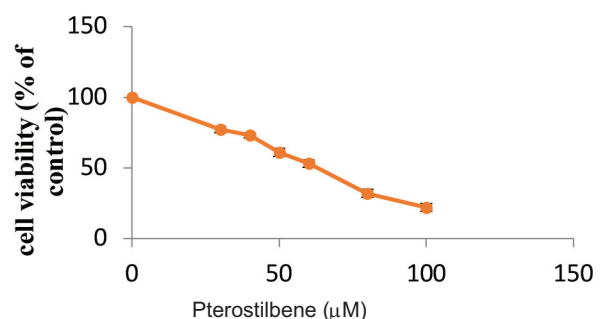
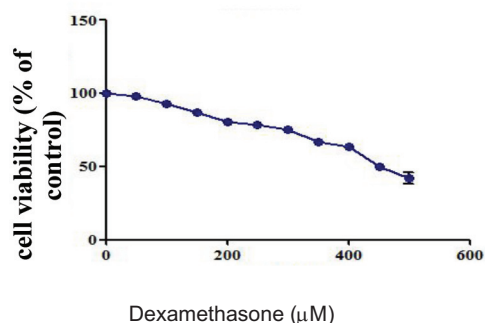


Figure 1. Effect of dexamethasone or pterostilbene on Jurkat cell viability. The cells were incubated with different concentrations of dexamethasone or PT for 48 h and MTS assay was performed to identify the viable cells. The cell viability decreased in a dose-dependent manner. All experiments were performed at least three times.

containing 5% CO₂ at 37°C.

Cell viability test

We applied MTS to evaluate cell viability according to a previously described method.¹⁶ We cultured Jurkat cells in a concentration of 104 cells per well in 96-well plates and kept them for 24 h. We treated the cells with different concentrations of PT (0-100 μM), dexamethasone (0.01- 1 IU/ml), or their combination for 48h. Following the incubation time, we added 20 μl of MTS to each well and incubated the cells for 3 h more at 37° C in a dark place. Finally, we measured the absorbance at 490-620 nm by an Elisa reader (stat fax-2100 awareness).

Apoptosis Assay

We measured apoptosis via flow cytometry with the FITC Annexin V propidium iodide (PI) Apoptosis Detection Kit (BD Pharmingen). Briefly, we implanted jurkat cells in a six-well plate in complete media for 24 h. Afterwards, the cells were treated with different concentrations of PT for 48 h, harvested, and washed twice in cold PBS. We resuspended 106 cells in 1 ml of 1X binding buffer. Next, we incubated 105 cells with 5 μl of FITC-labeled annexin V and PI (propidium iodide) at room temperature in darkness for 20 min; after that, we added 400 μl of 1X binding buffer. Ultimately, we evaluated the cells by flow cytometry. For comparison, we used the untreated sample as negative control.

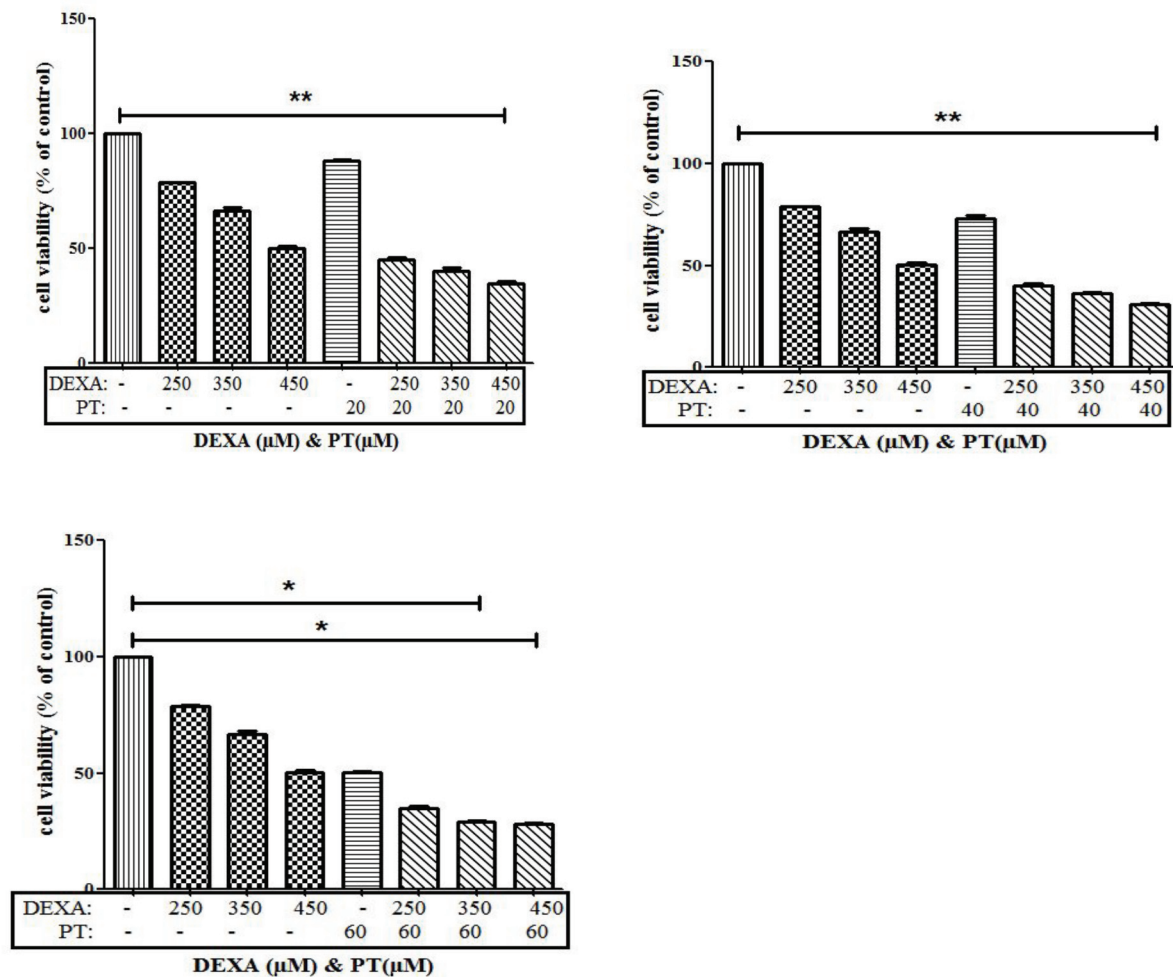


Figure 2. Cell viability in the presence of dexamethasone in combination with PT. Cell viability following 48 hours of incubation with different concentrations of dexamethasone alone or combined with different concentrations of PT was detected. Each experiment was performed three times or more and cell viability was compared with the controls. Cell viability decreased significantly when 450 μM of DEXA was combined with 20, 40, and 60 μM of PT or 350 μM of DEXA was combined with 60 μM of PT. (* indicates a significant difference with the controls and $P < 0.05$).

Statistical analysis

We repeated all experiments at least three times. Statistical analysis was performed by SPSS using Kruskal-Wallis multiple comparison and Dunn's test. We analyzed the inhibitory concentration of 50% (IC₅₀) value using probit analysis. We used Flowing software for flow cytometric analysis and GraphPad prism for graphs.

Results

Cell viability decreased in the presence of dexamethasone or PT

To determine the effect of dexamethasone on the viability of Jurkat cells, we incubated them for 48 h in the absence or presence of dexamethasone at different concentrations (0–500 μM). The ODs obtained from the absorption at 490 nm were adapted to a percentage. Figure 1 shows the reduction in cell viability with the

increase in concentration (dose-dependent) with an IC₅₀ (inhibition concentration) of 451.1±10.1 μM. Cell viability also decreased in a dose-dependent manner in the treatment by PT with an IC₅₀ of 60.97±3.36 μM.

PT enhanced the effect of dexamethasone on Jurkat cells

We assessed cell viability in the presence of different combinations of PT and dexamethasone. We detected a stronger cytotoxicity in all combinations compared with the separate use of each drug (Figure 2). Dexamethasone alone did not make any significant differences compared to the control at any of the used concentrations; however, 450 μM dexamethasone combined with 20, 40, and 60 μM PT as well as 350 μM dexamethasone in combination with 60 μM PT, resulted in a significant difference compared with the control.

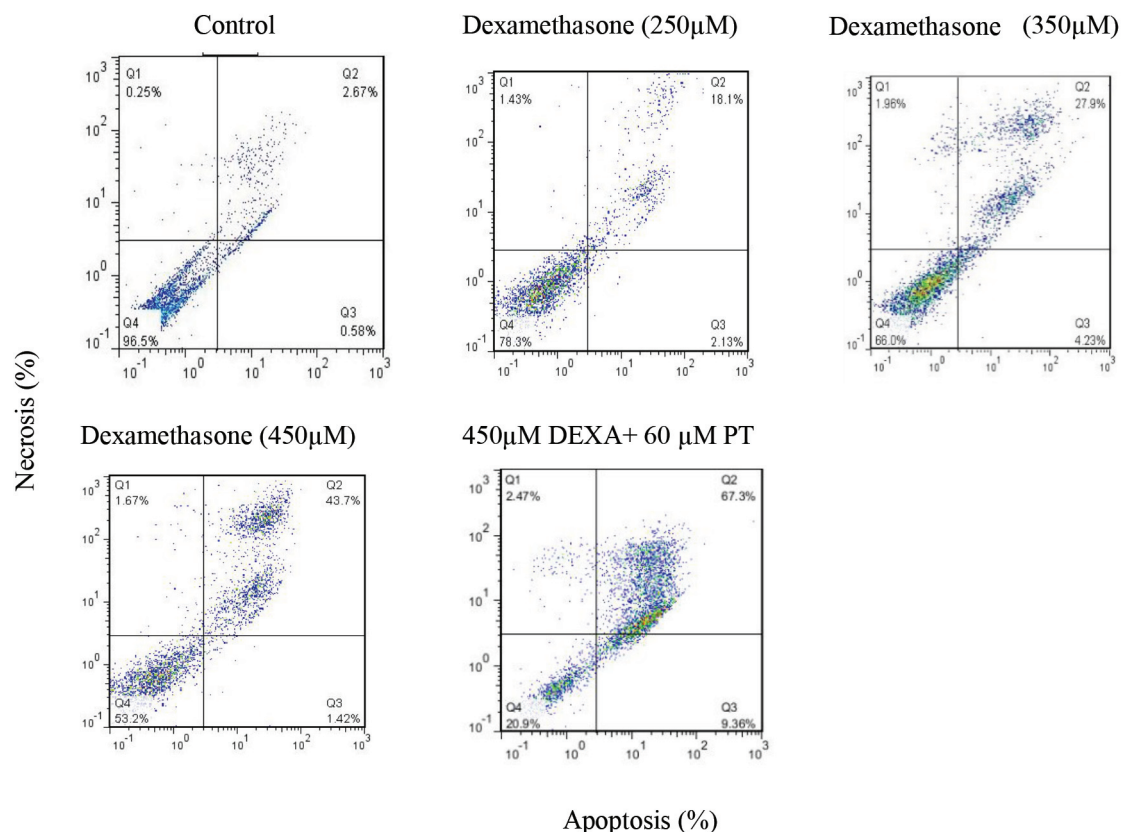


Figure 3. Flow cytometric analysis of apoptosis in the presence of dexamethasone alone or combined with PT. The cells were incubated for 48 h in the presence of different concentrations of dexamethasone alone or in combination with PT. In the presence of dexamethasone, apoptosis increased in a concentration-dependent manner; however, more apoptosis was observed when 450 μM concentration of dexamethasone was combined with 60 μM of PT (76.66 %).

PT or dexamethasone enhanced the apoptosis of Jurkat cells

We evaluated apoptosis induction via annexin-V/PI apoptosis detection kit. We incubated the cells for 48 h at various concentrations (46, 60, and 80 μM) of PT or dexamethasone (250, 350, and 450). At the above concentrations, PT induced apoptosis in 27, 49, and 60% of the cells in a dose-dependent manner. Apoptosis further increased in dexamethasone-treated cells in a dose-dependent pattern; however, it did not reach a significant level compared to the control.

PT potentiated the apoptosis induced by dexamethasone

Figure 3 shows the effect of combination treatment on apoptosis induction. Induction of apoptosis in treated cells augmented in certain combinations. 60 μM PT and 450 μM dexamethasone did not separately cause a significant difference in comparison with control; however, their combination significantly enhanced the apoptosis (Figure 4).

Discussion

In the present work, the decrease in cell viability by dexamethasone alone had no significant difference with the control; however,

the combination of 450 μM dexamethasone with 20, 40, and 60 μM PT as well as 350 μM dexamethasone with 60 μM PT significantly reduced the cell viability.

At 80 μM concentration, PT separately caused a significant apoptosis, but none of the used concentrations of dexamethasone alone caused such a difference. The combination of 450 μM dexamethasone with 60 μM PT led to a significant difference with the control; however, none of them exerted such an effect when applied separately.

The effect of PT on cell growth inhibition was studied on different cell lines. Wang et al. found the most inhibition after 72 hours with 50 μM concentration on breast cancer cells. They also observed cell cycle arrest at G1.¹⁷ Induction of apoptosis in cancer cells is a mechanism for preventing or treating cancer. Roslie et al. studied K562 chronic myelogenous leukemia cell line; they detected the highest apoptosis induction at 75 μM and 100 μM PT. This is comparable to our findings regarding lymphoblastic cells and more than what we observed with 100 μM atoposide. In their study, caspase-3 and 9 had the most increase after two hours of treatment with PT.¹⁸ At 75-100 μM , PT reduced cell viability in pancreatic cell lines and induced cell cycle

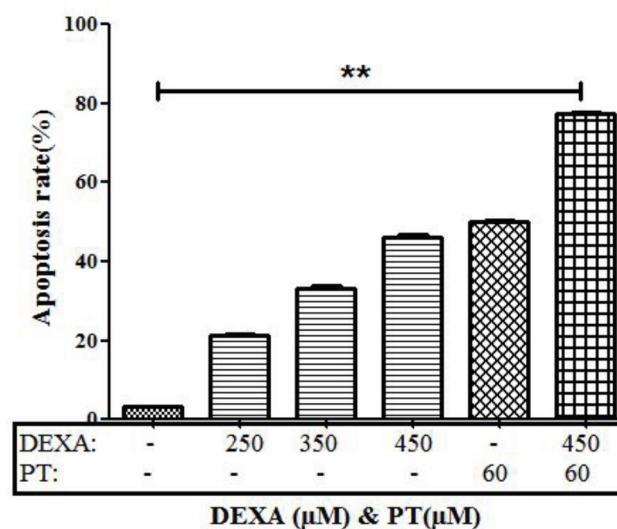


Figure 4. Comparative analysis of apoptosis in the presence of dexamethasone or in combination with PT. Jurkat cells were treated with different concentrations of dexamethasone alone or 450 μM in combination with 60 μM concentration of PT for 48 h; The cells were stained with FITC-conjugated annexin V and analyzed by flowcytometry. Untreated cells were considered as controls. A significant difference existed between the controls and 450 μM dexamethasone combined with 60 μM PT ($P < 0.01$).

arrest and caspases activation. IC₅₀ of pterostilbene is variable in different cell lines.¹⁹ PT effect was studied on seven cell lines, including human drug resistant myeloid cells. Tolomeo et al. reported that PT had a dose-dependent inhibitory effect on cell growth.²⁰

The combination effects of a PT and a chemotherapy reagent was also studied in some other cell lines. Mannal et al. demonstrated the combination effect of PT and tamoxifen on the proliferation inhibition and apoptosis induction of breast cancer cell lines.²¹ Their combination was significantly different with the separate application of tamoxifen. This effect was further observed in certain other combinations of a plant-derived component with a chemotherapy reagent. Soltani et al. found this effect in the treatment of lymphoblastic leukemia cells with a combination of thymoquinone and doxorubicine.²² We already demonstrated enhanced apoptosis and Fas expression both at mRNA level and surface, upon the treatment of the cells with a combination of gallic acid and L-asparaginase on these cells compared to the latter alone.²³ Here, for the first time, we showed the improved antiproliferative effect of dexamethasone combined with PT on lymphoblastic leukemia cells. The effect of this component on other T-cell lines and T-lymphoblasts needs to be further investigated.

Conclusion

Certain combinations of dexamethasone and PT (not dexamethasone alone) reduced the cell viability. This effect might be the result of apoptosis; however, other mechanisms may be involved. PT can be considered in combination chemotherapy of ALL; but, it is necessary to conduct more comprehensive studies on the leukemic cells of patients as well as in vivo in experimental models. The interaction of PT with other components of combination therapy needs to be further investigated.

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Conflicts of Interest

None declared.

References

- Gökbuğet N, Stanze D, Beck J, Diedrich H, Horst H-A, Hüttmann A, et al. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood*. 2012;120(10):2032-41.
- Belver L, Ferrando A. The genetics and mechanisms of T cell acute lymphoblastic leukaemia. *Nat Rev Cancer*. 2016;16(8):494-507. doi: 10.1038/nrc.2016.63.
- Roti G, Stegmaier K. New Approaches to target T-ALL. *Front Oncol*. 2014;4:170. doi: 10.3389/fonc.2014.00170.
- Senthilkumar R, Chen BA, Cai XH, Fu R. Anticancer and multidrug-resistance reversing potential of traditional medicinal plants and their bioactive compounds in leukemia cell lines. *Chin J Nat Med*. 2014;12(12):881-94. doi: 10.1016/S1875-5364(14)60131-X.
- Asgari S, Rafieian-Kopaei M, Pourgheysari B, Ansari Samani R, Deris F, Shahinfard N, et al. *Allium hirtifolium* Boiss: Radical scavenging property and the lowering effects on blood fibrinogen and factor VII. *Life Science Journal*. 2012;9(3):1793-8.
- Sourani Z, Pourgheysari B, Beshkar P, Shirzad H, Shirzad M. Gallic acid inhibits proliferation and induces apoptosis in lymphoblastic leukemia cell line (C121). *Iran J Med Sci*. 2016;41(6):525-30.
- Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. *Nutr J*. 2016;15(1):99.
- Athar M, Back JH, Kopelovich L, Bickers DR, Kim AL. Multiple molecular targets of resveratrol: Anticarcinogenic mechanisms. *Arch Biochem Biophys*. 2009;486(2):95-102.
- Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. Plant foods and herbal sources of resveratrol. *J Agric Food Chem*. 2002;50(11):3337-40.
- Kapetanovic IM, Muzzio M, Huang Z, Thompson TN, McCormick DL. Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother Pharmacol*. 2011;68(3):593-601.
- Hasiah A, Ghazali AR, Weber J, Velu S, Thomas N, Inayat Hussain S. Cytotoxic and antioxidant effects of methoxylated stilbene analogues on HepG2 hepatoma and Chang liver cells: Implications for

- structure activity relationship. *Hum Exp Toxicol*. 2011;30(2):138-44.
12. Moon D, McCormack D, McDonald D, McFadden D. Pterostilbene induces mitochondrially derived apoptosis in breast cancer cells in vitro. *J Surg Res*. 2013;180(2):208-15.
 13. Schneider JG, Alosi JA, McDonald DE, McFadden DW. Pterostilbene inhibits lung cancer through induction of apoptosis. *J Surg Res*. 2010;161(1):18-22.
 14. Pei HL, Mu DM, Zhang B. Anticancer activity of pterostilbene in human ovarian cancer cell lines. *Med Sci Monit*. 2017;23:3192-9.
 15. Ringler I, West K, Dulin W, Boland EW. Biological potencies of chemically modified adrenocorticosteroids in rat and man. *Metabolism*. 1964;13(1):37-44.
 16. Ghasemi-Pirbaluti M, Pourgheysari B, Motaghi E, Askarian Dehkordi N, Surani Z, Shirzad M, 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.
 17. Wang Y, Ding L, Wang X, Zhang J, Han W, Feng L, et al. Pterostilbene simultaneously induces apoptosis, cell cycle arrest and cyto-protective autophagy in breast cancer cells. *Am J Transl Res*. 2012;4(1):44.
 18. Roslie H, Chan KM, Rajab NF, Velu SS, Abd SAIAS, Bunyamin I, et al. 3, 5-Dibenzoyloxy-4'-hydroxystilbene induces early caspase-9 activation during apoptosis in human K562 chronic myelogenous leukemia cells. *J HerbMed Pharmacol*. 2012;37(1):13-21.
 19. Mena S, Rodriguez ML, Ponsoda X, Estrela JM, Jäättela M, Ortega AL. Pterostilbene-induced tumor cytotoxicity: a lysosomal membrane permeabilization-dependent mechanism. *PLoS One*. 2012;7(9):e44524. doi: 10.1371/journal.pone.0044524.
 20. Tolomeo M, Grimaudo S, Di Cristina A, Roberti M, Pizzirani D, Meli M, et al. Pterostilbene and 3'-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. *Int J Biochem Cell Biol*. 2005;37(8):1709-26.
 21. Mannal P, McDonald D, McFadden D. Pterostilbene and tamoxifen show an additive effect against breast cancer in vitro. *Am J Surg*. 2010;200(5):577-80.
 22. Soltani A, Pourgheysari B, Shirzad H, Sourani Z. Antiproliferative and apoptosis-inducing activities of thymoquinone in lymphoblastic leukemia cell line. *Indian J Hematol Blood Transfus*. 2017;33(4):516-24.
 23. Sourani Z, Shirzad H, Shirzad M, Pourgheysari B. Interaction between gallic acid and asparaginase to potentiate anti-proliferative effect on lymphoblastic leukemia cell line. *Biomed Pharmacother*. 2017; 96:1045-54.