

Anti-proliferative Effects of Curcumin in Human Acute Lymphoblastic Leukemia Cell Line

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is one of the most common cancers among children. Although there have been tremendous treatments, none of them have led to a precise cure. The use of herbal medicines which are safe and non-toxic have been demonstrated in this study. Curcumin is a polyphenol, hydrophobic product that is derived from turmeric plant. Curcumin has anti-toxic, anti-bacterial, anti-inflammatory, anti-cancer and anti-apoptosis properties. In recent years, extensive researches have been performed over the use of curcumin on cancers. In this study, CCRF-CEM cell line has been treated by curcumin. Rate of cytotoxicity of curcumin and the viability of the cells after treatment were evaluated by MTT assay and flow cytometry analysis. When different concentrations of curcumin were used upon the CCRF-CEM cell line at different times, it was found that curcumin effect depended on dose and time pattern. The results revealed that curcumin could induce apoptosis in CCRF-CEM cell line of acute lymphoblastic leukemia.

Keywords: Anticancer; Curcumin; Ischemia; Human Acute Lymphoblastic leukemia

INTRODUCTION

Cancer develops due to genetic mutations in oncogenes and tumor suppressor genes, and has been regarded as one of the major causes of death in the world. [1, 2]. One of the cancers prevalent in people aged under twenty is acute lymphoblastic leukemia caused by malignancy of T or B cells [3, 4]. Until today various treatments have been carried out on cancers, and yet not often have they lead to the prevention of tumor recurrence. Natural compounds with safety and lack of toxicity have resulted in renewed hope in the treatment of cancer [5]. Curcuma Longa is a perennial plant and a member of the ginger family which is a curcuminoid most abundant in Turmeric [6]. For the first time, curcumin was extracted by Vogel in 1815 [7, 8, and 9]. The molecular formula of curcumin is $C_{21}H_{20}O_6$ [9]. Since 1987, the National Cancer Institute has conducted experiments on over 1000 different materials to achieve the anticancer reaction. Curcumin is one of the materials recognized to be effective for cancer research. Due to the application of curcumin in the diet for centuries,

it can be characterized as completely safe for pharmaceutical use [6, 10]. Studies on curcumin showed that it has anti-toxic, anti-bacterial, anti-viral, anti-inflammatory, anti-malarial and antioxidant properties [6, 11]. According to clinical findings first reported by Kuttan et al, curcumin has anti-cancer activity as well as induced apoptosis. According to the clinical studies, the usage of curcumin up to 12 g/ day is easily tolerated [7, 10]. Curcumin with its variety of functions and potent anticancer activity can prevent progression, metastasis and angiogenesis in tumors. Unlike the other drugs, curcumin has a variety of targets such as proteins, transcription factors, growth factors, receptors, cytokines, enzymes and etc. [12]. As curcumin can react with 30 different proteins, it is able to induce apoptosis due to multiple signaling pathways [13, 11]. Curcumin can inhibit both NF- κ B and AP-1 (activator protein 1) which are transcription factors and increase the expression of genes involved in inflammation, proliferation and cell growth in many cancers. [6]. Curcumin can also inhibit the activity of a tyrosine kinase receptor

named HER2/neu and other epidermal growth factor receptors and suppresses breast cancer cells [14]. (human epidermal growth factor receptor2, c-erbB-2 and human EGF receptor 2 are the other names of HER2/neu). According to some studies, curcumin induces apoptosis of breast cancer cells as well as leukemia [15, 16]. In this experimental study, curcumin has been used to treat the CCRF-CEM cell lines of acute lymphoblastic leukemia, where the apoptotic activity of curcumin was investigated.

MATERIALS AND METHODS

The required purchase was done as follows:

Curcumin from Riedel-de Haen. Dimethyl sulfoxide [DMSO]; MTT from Sigma America; CCRF-CEM cell line of acute lymphoblastic leukemia from Pasteur Institute of Iran; RPMI 1640 medium and pen / Strep Caisson America, and FBS from Gibco America.

Cell culture

CCRF-CEM cell lines were cultured in RPMI-1640 containing 10 % FBS, 1% pen/strep under a humid condition [37 °C, 5 % CO₂, 95 % air].

MTT assay

MTT assay is one of the in vitro widely used ways to evaluate the relative number of the viable cells after treatment with drugs. The mitochondrial succinate dehydrogenase enzymes in living cells can revive the yellow MTT to a purple color which can be measured by ELISA reader at 570nm. . 1×10⁴ CCRF-CEM cells were plated in 96-well plates. After 24 hours, the cells were treated using 6.25, 12.5, 25 and 50 µg/ml concentrations of curcumin in RPMI medium containing 1% Pen Strep separately. Each concentration was considered in 8 wells. The plates were incubated at 37°C for 24, 48 and 72 hours. 10µL of MTT (5mg/mL) was added to each well and the plates were incubated at 37°C. After 2 to 4 hours, 100 µl DMSO was added to each well and mixed till the pellets were dissolved thoroughly. The absorbance was read at

OD₅₇₀ and the cytotoxicity was calculated by the following formula:

$$\text{Cytotoxicity \%} = 1 - \frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}} \times 100$$

$$\text{Viability \%} = 100 - \text{Cytotoxicity \%}$$

Flow cytometry analysis

To evaluate the rate of cytotoxicity of curcumin and the viability of the cells after treatment, flow cytometry analysis was performed. For this purpose the annexin V/propidium iodide assay was performed according to the manufacturer's recommendation. Briefly, CCRF-CEM cells were plated into a 12-well plates and incubated for 24, 48 and 72 hours with 50 µg/ml concentration of curcumin. The cells were collected, centrifuged and washed with calcium buffer. Annexin V [10 µl] was added to the cells and were incubated on ice for 20 minutes in the darkness. Then the cells were centrifuged and washed with calcium buffer again. PI [10 µl] was added to the cells and incubated on ice for 10 minutes. At the end, calcium buffer [300 µl] was added and the mixture was analyzed through flow cytometer.

RESULTS

Vital capacity test after treatment with curcumin by MTT assay

The results of curcumin effects on CCRF-CEM cell lines were analyzed by MTT assay in different times and concentrations, and are shown in Figure 1. IC₅₀ concentrations of curcumin at 24, 48 and 72 hours, were about 50, 25 and less than 6.25 µg/ml respectively.

Pro-apoptotic capacity test after treatment with curcumin by flow cytometry assay

The results of the apoptotic effects of 50 µg/ml curcumin on CCRF-CEM cell line after 24, 48 and 72 hours are shown in Figure 2. According to the obtained results, the percentage of treated living cells with curcumin is significantly different with control cells.

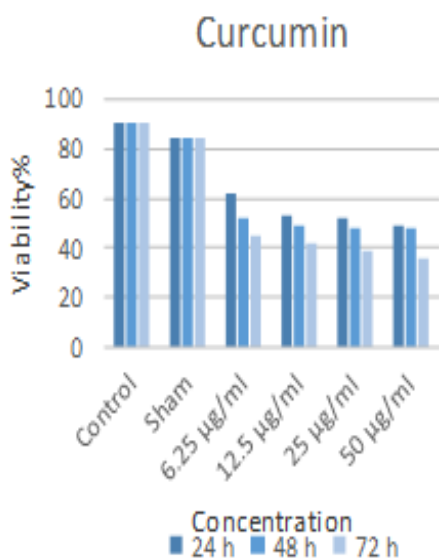


Figure 1. Viability effects of curcumin on CCRF-CEM cell line

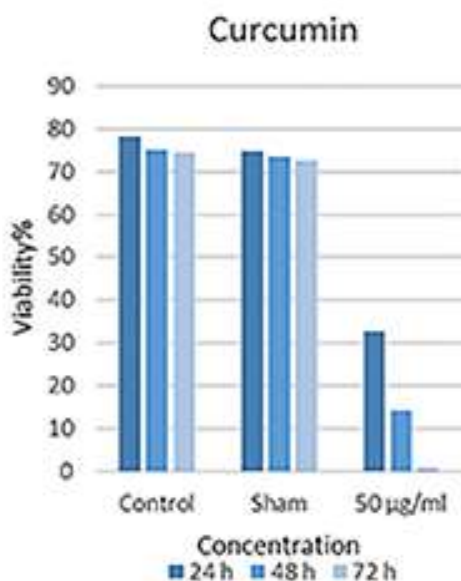


Figure 2. Apoptotic effects of curcumin on CCRF-CEM cell line by flow cytometry

DISCUSSION

Cancer has been regarded as a significant health threat and the second cause of mortality in developed countries [17]. BCR-ABL¹ occurs in

¹ a reciprocal translocation, t (9; 22) (breakpoint cluster region gene locus-Abelson leukemia, abl gene locus)

adults with acute lymphoblastic leukemia more than children. Unlike the chronic myeloid leukemia, the disease does not improve with chemotherapy and bone marrow transplantation [3]. Despite the tremendous progress in treatment of cancer, the problem of multidrug resistance, remains a real problem in blood, breast, and ovaries cancers and etc. [17]. First, Natural products have been used to cure patients with low toxicity making no sensitivity.

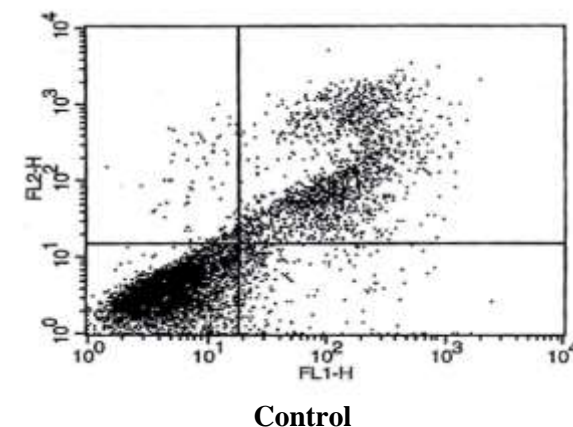
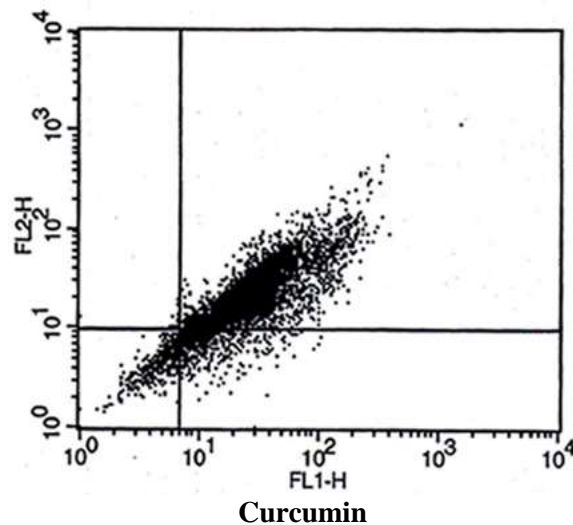


Figure 3. Apoptotic effects of curcumin (50 µg/ml) on CCRF-CEM cell line at 48h by flow cytometry

Curcumin as a natural active polyphenol has anti-cancer effects and has been used in the treatment of various diseases including diarrhea, jaundice, ulcers, etc [2]. Curcumin through its different signaling pathways has different molecular

targets and regulates cell proliferation and apoptosis [1]. The caspases, a family of cysteine-aspartate proteases, plays an important role in necrosis, apoptosis and inflammation. According to the numerous reports, curcumin promotes apoptosis by inducing Caspases [18]. It is demonstrated that curcumin can be effective on stem cell cancer in multiple pathways by Klonisch et al. and Kakarala et al. in 2008 and 2010 [19, 20]. Curcumin inhibits the main characteristics of cancers that increase expression of the biomarkers, such as CD133, CD44, CD166 and ALDH1. This can change the morphology of stem cells cancer [19, 20]. Anad et al and Shehzad et al in 2008 and 2010 found that curcumin is effective on several genes linked to breast cancer, such as NF- κ B, AP-1, COX-1, VEGF, cyclin E, factor fibroblast growth (FGF), IL-6, IL-11, MMP-9 and MMP-13 and also on the up-regulation on P21 and P27 [21, 22]. In 2011, while doing researches on patients with colorectal cancer, it was demonstrated that curcumin increases the amount of P53, which conduces to improve patients [23]. Aggarwal et al. and Dorai et al. in 2008 and 2001 while

working on the patients with prostate cancer demonstrated that treatment of those patients with curcumin inhibited angiogenesis and proliferation [24, 25].

Watson et al (2001) during treatment of the patients with ovarian cancer by curcumin found that Bcl₂ expression and Survivin, which are anti-apoptotic proteins were decreased; it also created a down regulation of Akt signaling and an activation of p38-MAPK [26].

CONCLUSION

We used curcumin on CCRF-CEM cell line in acute lymphoblastic leukemia to find out growth inhibition and induction of apoptosis. The results of this study showed that curcumin can inhibit cell growth and can also induce apoptosis in CCRF-CEM cell lines of acute lymphoblastic leukemia.

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“The authors declare no conflict of interest”

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