Cytotoxicity of Curcumin against Leukemic Cell Lines via Apoptosis Activity

Farah J. Hashim, Muayed S. Shawkat and H. Al-Jewari

Department of Biotechnology, College of Science, Baghdad University, Baghdad, Iraq

Ibn Al-Haitham Education College, Baghdad University, Baghdad, Iraq

Abstract: The aimed of this study was to evaluate curcumin as anticancer agent. The apoptogenic and cytotoxic activity of curcumin were investigated against two human leukemic cell lines-U937 and Molt4. Cytotoxic activity was performed by using [3-[4, 5-dimethylthiazol-2-yl] 2, 5 diphenyltetrazolium bromide] MTT assay. Also 4, 6-diamidino-2-phenylindole di-hydrochloride [DAPI] staining was used as a DNA-binding fluorochrome for in vitro apoptosis detection. Our result showed that curcumin have cytotoxic activity to both U937, Molt4 cell lines with 9.25 and 6.59/mL IC50 for U937 and Molt4 respectively. Otherwise curcumin was found to possess apoptotic activities which were 80.02 and 55.06% for U937 and Molt4, respectively compare with 90 and 88% against U937 and Molt4 in melphalan and the process of apoptosis was characterized by several morphological changes such as nuclear fragmentation. In summary, curcumin possessed anticancer activity by inhibition cell growth and metabolic activity of the leukemic cells and showed characteristic features of apoptosis. Further biological testing on the efficacy of purified curcumin will be conducted to get better understand of the mechanism of anticancer activity.

Key words: Apoptosis, curcumin, cytotoxicity, DAPI stain, IC50, leukemic, MIL assay

INTRODUCTION

Now-a-days beneficial aspects of dietary constituents of plant origin have been extensively studied (Shukla et al., 2002). Phytochemicals are bioactive non-nutrient components of various plant parts, such as seeds, leaves, and rhizomes. Recent epidemiological and preclinical testing has revealed the great potential of phytochemicals in combating cancer and other chronic diseases that result from oxidative stress induced by free radicals. (Thangapazham et al., 2006)

Curcumin, a natural yellow pigment present in the rhizomes of turmeric (Curcuma longa) and related species and used as a spice, has a wide array of pharmacological and biological activities many years (Jagetia and Aggarwal, 2007; Rithaporn et al., 2003). curcumin reduces blood cholesterol, inhibits platelet aggregation, suppresses symptoms associated with type II diabetes, rheumatoid arthritis, Alzheimer, inhibits HIV replication, enhances wound healing, protects from liver injury, increases bile secretion, and protects from pulmonary toxicity and fibrosis (Aggarwal et al., 2003). Otherwise, it has been considered as one of the most promising chemopreventive agents against a variety of human cancers including colon, duodenal, stomach, prostate, leukemia and breast cancers (Kong et al., 2009; Thangapazham et al., 2006), cytotoxicity of curcumin against cancer cell lines can investigated by using MTT assay which is cell viability assay often used to determine cytotoxicity following exposure to toxic substances (Fotakis and Timbrell, 2006).

Human leukemia is one of the commonly diagnosed neoplasms and the major leading cause of human death. Although melphalan is a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents, it slows or stops the growth of cancer cells in any body (Facon et al., 2007). The development of drug resistance and severe side effects of standard anticancer drugs necessitate the search for novel treatment options for this disease (Kong et al., 2009; Lin and Shiau, 2001) demonstrated that curcumin induces apoptosis in several tumor cell lines which detected by various tools, DAPI staining (a DNA-binding fluorochrome) one of these tools (Zainol et al., 2009). The purpose of the present study was to investigate the cytotoxic and apoptogenic activities of curcumin standard in human leukemic cell lines.

MATERIALS AND METHODS

Cell lines and chemicals: The human acute monocyte leukemia (U937) cell line and human acute lymphoblastic leukemia (Molt4) were purchased from the American Type Culture Collection (ATCC, Rockville, MD). And maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, and Penicillin-streptomycin (1% v/v) (Gibco, UK). The cells were kept at 37°C in an
Results and Discussion

Cytotoxicity of curcumin using MTT assay: Compounds that block or suppress the proliferation of tumor cells have potential as anticancer agents. Phenolic compounds have widespread occurrence in nature and were consumed by humans through diet containing it as fruits, vegetables and beverages. These minor non-nutrient dietary factors elicit considerable chemopreventive activity against carcinogenesis (Roy et al., 2002). Moreover, curcumin has been shown to inhibit the proliferation of a wide variety of tumor cells, including leukemia (Aggarwal et al., 2005).

Table 1: Cytotoxicity results of curcumin in U937 and Molt4 cell lines

<table>
<thead>
<tr>
<th>Concentration of curcumin µg/mL</th>
<th>Curcumin inhibition % against Molt4 (mean±SE)</th>
<th>Curcumin inhibition % against U937 (mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0±0.0</td>
<td>15.13±4.70</td>
</tr>
<tr>
<td>4</td>
<td>0.30±0.03</td>
<td>26.07±4.30</td>
</tr>
<tr>
<td>8</td>
<td>77.35±1.93</td>
<td>59.20±0.45</td>
</tr>
<tr>
<td>16</td>
<td>82.32±1.31</td>
<td>74.45±0.50</td>
</tr>
<tr>
<td>32</td>
<td>85.72±1.1</td>
<td>88.91±0.36</td>
</tr>
</tbody>
</table>
cell lines under study might indicate the presence or absence of specific cellular receptors in each type of cell lines, making the cells respond in different manners at same concentration. Moreover, may be the metabolic pathways of cancerous cells in response to each treatment differed from one line to another.

Curcumin may inhibit proliferation of leukemic cells by arresting them in various phases of the cell cycle and by inducing apoptosis (Tamvakopoulos et al., 2007).

2-Apoptosis test by DAPI staining: To confirm the induction of apoptosis by Curcumin, U937 and Molt4 cell lines were treated with various concentrations of curcumin then stained with DAPI stain.

Observation under fluorescent microscope revealed that treated leukemic cells with Curcumin stained with 4, 6-diamidino-2-phenyl indole dihydro chloride (DAPI) staining and had orange-red fluorescent color which gave bright green fluorescent color compared with untreated.

![Images of untreated and treated cells](image_url)

**Fig. 1:** Fluorescence microscopic images of untreated control U937 (a), Molt4 cells (b) and standard Curcumin treated U937 (c), Molt4 cells (d) and Melphalan (which used as standard reference anti-cancer drug) treated U937 (e), Molt4 cells (f). The control cells were with intact nucleus and gave bright blue fluorescence whereas treated cells showed intense fragments of nucleus as signs of apoptosis by DAPI staining.
cells (control), indicating the fact that the treatment with curcumin brought about apoptotic changes in the cells like nuclear fragmentation compared with control cells with intact nucleus, (Fig. 1).

Results represents that curcumin was found to possess apoptotic activities which were 80.02 and 55.06% for U937 and Molt4, respectively compare with 90 and 88% against U937 and Molt4 as clearly chromatin condensation and nuclear fragmentation of treated cells.

Curcumin described to efficiently induce apoptosis in various cell lines (Duvoix et al., 2005). Curcumin (diferuloyl methane) was previously shown to induce apoptosis in malignant cancer cell lines including leukemic cell lines (Roy et al., 2002).

Ghosh et al. (2009) reported that curcumin exhibited notable anti-proliferative activity towards lymphoblast leukemic cells by induce DNA damage in cancer cells, finally leading to the apoptosis of cancer cells. It has been suggested that curcumin induces apoptosis in tumor cells by mitochondria-dependent mechanisms (Aggarwal et al., 2003; Reuter et al., 2008; Woo et al., 2003) suggested that curcumin activate cytochrome c caspase-3.

In the last years more and more studies demonstrated the increased apoptotic effect of curcumin combined with chemotherapeutic drugs compared to the effect of chemotherapeutic drugs alone. Because it is becoming obvious that the molecular bases for most common diseases are far more complex, this argues against the use of drugs that center on single-target or single-drug approaches. Therefore it is becoming imperative to adopt a multi-target based drug development paradigm for the treatment of complex human diseases that work by different mechanisms of action, thereby leading them to decrease the probability that cancer cells will develop resistance against chemotherapeutic drugs (Reuter et al., 2008).

**CONCLUSION**

In conclusion, our results demonstrated that Curcumin a natural yellow pigment of *Curcuma longa* rhizomes have inhibition effects which is sufficient to inhibit proliferation of U937 and Molt4 cell lines. Otherwise results showed that Curcumin induces apoptosis in both leukemic cell lines compared to melphalan. The results suggest that the apoptotic activity of Curcumin may contribute to their claimed anticancer property.

**REFERENCES**


