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**ORIGINAL ARTICLE / ÖZGÜN MAKALE**

# COENZYME Q0 INHIBITS CELL PROLIFERATION AND MODULATES MAPK AND AKT SIGNALLING PATHWAYS IN HUMAN CHRONIC MYELOID LEUKEMIA K562 CELLS

*KOENZİM Q0 İNSAN KRONİK MYELOİD LÖSEMİ K562 HÜCRELERİNİN PROLİFERASYONUNU ENGELLER VE MAPK VE AKT SİNYAL YOLAKLARINI MODÜLE EDER*

**Ecem KAYA-SEZGİNER[1], Ali YAPRAK[1], Arzu Zeynep KARABAY[1]***

[1]Ankara University Faculty of Pharmacy, Department of Biochemistry, 06560, Ankara, Turkey

## ABSTRACT

**Objective:** *This study evaluated the antiproliferative and pro-apoptotic effects of coenzyme Q0 (CoQ0) in human chronic myeloid leukemia K562 cell line.*
**Material and Method:** *The cytotoxic effect of CoQ0 on human chronic myeloid leukemia cell line, K562 was determined by MTT test. The activity of caspase-3, expression of proteins involved in apoptosis, MAPK and AKT signaling pathways were determined with enzymatic assay and western blot analysis, respectively.*
**Result and Discussion:** *Results showed that CoQ0 inhibited cell viability of K562 cells at 5 µM and higher concentrations and Bax protein expression was significantly decreased at 12.5 µM concentration of CoQ0. However, CoQ0 did not significantly affect caspase 3 activity and Bcl-2 protein expression. p-c-Raf (Ser259) protein expression was significantly decreased at 12.5 µM of CoQ0. Treatment with 10 µM of CoQ0 induced significantly phosphorylation of p38 MAPK and 12.5 µM CoQ0 caused a nonsignificant decrease in p-ERK1/2 protein expression in K562 cell line. Interestingly, in K562 cells, phosphorylation of Akt (Ser473) was diminished at 12.5 µM of CoQ0, with no change observed in p-Akt (Thr308) protein expression among groups. In conclusion, CoQ0 inhibited cell proliferation and suppressed phosphorylation of c-Raf (Ser259), Akt (Ser473), but not ERK1/2 in K562 cells. There is still a need for new insights into the anticancer mechanisms of CoQ0 and develop treatment strategies for chronic myeloid leukemia.*
**Keywords:** *Chronic myeloid leukemia, coenzyme Q0, K562*

* **Corresponding Author / Sorumlu Yazar:** Arzu Zeynep Karabay
**e-mail / e-posta:** zeynepkarabay@yahoo.com, **Phone / Tel.:** +903122033057

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## ÖZ

**Amaç:** *Bu çalışma, insan kronik miyeloid lösemi K562 hücre hattında koenzim Q0'ın (CoQ0) antiproliferatif ve proapoptotik etkilerini değerlendirmiştir.*

**Gereç ve Yöntem:** *CoQ0'ın insan kronik miyeloid lösemi K562 hücre hattındaki sitotoksik etkisi, MTT testi ile belirlendi. Kaspaz-3 aktivitesi, apoptozis, MAPK ve AKT sinyal yolağı ile ilişkili proteinlerin ekspresyonu sırasıyla enzimatik analiz ve western blot analizi ile belirlendi.*

**Sonuç ve Tartışma:** *Sonuçlar, CoQ0'ın K562 hücre canlılığını 5 μM ve daha yüksek konsantrasyonlarda inhibe ettiğini ve Bax protein ekspresyonunu, 12.5 μM konsantrasyonunda önemli ölçüde azalttığını göstermiştir, ancak CoQ0 kaspaz 3 aktivitesini ve Bcl-2 protein ekspresyonunu önemli ölçüde etkilemedi. p-c-Raf (Ser259) protein ekspresyonu, 12.5 μM CoQ0'da önemli ölçüde azaldı. K562 hücre hattında, 10 μM CoQ0, p38 MAPK'nın fosforilasyonunu önemli ölçüde indükledi ve 12,5 μM CoQ0, p-ERK1/2 protein ekspresyonunda anlamlı olmayan bir azalmaya neden oldu. İlginç bir şekilde, 12.5 μM CoQ0 K562 hücrelerinde Akt (Ser473) fosforilasyonu azalttı, ancak p-Akt (Thr308) protein ekspresyonunda gruplar arasında herhangi bir farklılık gözlenmedi. Sonuç olarak, CoQ0, K562 hücrelerinin proliferasyonunu inhibe etti ve c-Raf (Ser259), Akt (Ser473) fosforilasyonunu baskıladı, ancak ERK1/2 fosforilasyonuna etki etmedi. CoQ0'ın antikanser etkisinin altında yatan moleküler mekanizmalara yeni bakış açıları sağlamak ve kronik miyeloid lösemi tedavi stratejilerini geliştirmek için daha fazla araştırmaya hala ihtiyaç bulunmaktadır.*


**Anahtar Kelimeler:** *Koenzim Q0, kronik miyeloid lösemi, K562*

## INTRODUCTION

Chronic myeloid leukemia (CML) is a type of myeloproliferative disorder where an abnormality in chromosome structure, known as t(9;22), leads to the formation of the Philadelphia (Ph) chromosome and Bcr-Abl chimeric oncoprotein [1]. Bcr-Abl oncoprotein enhances cell growth and proliferation of leukemia cells and inhibits apoptosis [2]. The vast majority of individuals diagnosed with CML exhibits a positive response to imatinib, a Bcr/Abl kinase competitive inhibitor. However, there exists a possibility for CML patients to develop clinical resistance to imatinib or experience only temporary remissions despite ongoing treatment [3-5]. Therefore, an alternative therapeutic strategy for CML is required to prevent the progression of the disease to more advanced stages and reduce the risk of death.

Coenzyme Q (CoQ0) is a biomolecule which accumulates predominantly in mitochondria [6,7]. The strong toxicity of CoQ0 has been demonstrated in several cancer cell lines [6,8], and CoQ0 stimulates secretion of insulin by pancreatic islets [9], and has anti-angiogenic and anti-inflammatory properties [10,11]. Researchers have demonstrated that CoQ0 displays potent cytotoxic effects against triple negative breast cancer cell line (MDA-MB-231) by induction of apoptosis and cell cycle arrest, inhibition of metastasis and epithelial-mesenchymal transition [6,12]. CoQ0 significantly induced cell cycle arrest, apoptosis, and inhibited metastasis in melanoma cell lines [13]. In MCF-7 cell line, CoQ0 enhanced ultraviolet B-induced apoptosis [14]. Another CoQ analogs (CoQ2 and CoQ4) induced apoptosis in mutated human acute lymphoblastic leukemia BALL-1 cells [15]. CoQ0 significantly induced cell death in cancerous rat liver MH1C1 cells [16].

Induction of cancer cell apoptosis, the most common form of programmed cell death, is one of the main chemotherapeutic approaches [17]. Activation of caspase-3 is considered to be a main characteristic of apoptosis [18]. Bcl-2 family members (anti-apoptotic proteins Bcl-2 and Bcl-xL and pro-apoptotic proteins Bax, Bak, and Bid) regulate mitochondrial pathway of apoptosis [19,20].

The mitogen-activated protein kinase (MAPK) pathway modulates cell growth and differentiation, apoptosis, migration, survival, and death through the phosphorylation of target proteins [21-23]. MAPKs are comprised of three main subfamilies: the p44/42 (extracellular signal-regulated kinase, ERK1/2), c-Jun N-terminal kinases (JNKs) and p38 MAPKs [24,25]. Raf serine/threonine protein kinase promotes the activation of ERK1/2 which phosphorylates several substrates and modulates different transcription factors and also gene expression [26,27]. Therefore, targeting Raf and ERK1/2 pathway has been considered as potential therapeutic targets in the development of pharmacological agents for anti-cancer treatment [28,29]. Elevated ERK activity in human tumors was

suggested to be a marker of tumor progression [28-30] and decreased ERK1/2 phosphorylation may suppress cancer cell invasion [31].

Enhanced phosphoinositide 3-kinase (PI3K)/Akt activity is associated with poor clinical outcomes in hematological malignancies [32,33], indicating that inhibition of PI3K/Akt activation could be a promising in the management of leukemia [34,35]. Akt inhibits apoptosis by phosphorylation by initiation of multiple additional conversions involved in cell survival or apoptosis [36,37].

The anticancer effect of CoQ0 on chronic leukemia cancer cells was not reported previously. Therefore, we aimed to investigate the cytotoxic and apoptotic effect of CoQ0 on chronic myeloid leukemia K562 cell line through MAPK and AKT signaling pathways modulation.

## MATERIAL AND METHOD

### Cell Proliferation Assay

K562 cells were seeded in 96 wells plate at a density of $2x10^4$ cells/well and treated with CoQ0 between 1-20 μM during 24 h at 37°C. Then, MTT solution (5 mg/ml) was added to each well, and the plates were incubated for 2 h at 37°C in the dark. After incubation, the formed formazan crystal in each well was dissolved by MTT lysis buffer (20% SDS/50% dimethylformamide) and absorbance value of each well was measured by a spectrophotometer (Molecular Devices Co., CA, USA) at 570 nm. The final DMSO concentration did not exceed 0.25% in the incubation medium.

### Caspase-3 Activity Assay

The activity of caspase-3 was determined by colorimetric assay kit (Abcam Inc., UK). K562 cells with and without CoQ0 treatment (10 and 12.5 μM) were lysed in ice-cold lysis buffer and centrifuged at 10000×g for 1 min. The supernatant was collected to obtain total protein of each sample. Firstly, reaction buffer was added to cell lysates which include equal amounts of protein. Next, caspase-3 colorimetric DEVD-pNA substrate was added to the mixture. Following incubation at 37°C for 2 h, the resulting colorimetric product was measured with a microplate reader (Multiskan GO, Thermoscientific, Waltham, MA, USA) at 405 nm.

### Western Blot Analysis

K562 cells were harvested and lysed with a lysis buffer. Cell lysates were centrifuged at 8000 rpm at 4°C for 30 min. Protein amount was determined via the Bradford method, and equal amounts of denatured proteins were subsequently subjected to electrophoresis and transfer to a PVDF membrane for 60 min at 100 volts. Blocking of non-spesific binding was performed by incubation with non-fat dry milk (5% w/v) in phosphate buffer saline for 1h. Then, membranes were incubated overnight at 4°C with specific primary antibodies (1:1000), including Bax, Bcl-2, p-c-raf (Ser259), p-p38 MAPK, p-ERK1/2, p-Akt (Ser473), p-Akt (Thr308). The membranes were washed with 1x PBS for 10 min, and then source matched secondary antibodies in PBS-T were added for 1 h at room temperature. Next, the blots were visualized using a chemiluminescence substrate (ECL) and Odyssey Fc system (LI-COR Biosciences, Lincoln).

### Statistical Analysis

The data were displayed as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's Post Hoc test was performed for statistical analysis via GraphPad Prism 7 (San Diego, CA). $p<0.05$ was considered as the minimum level of significance.

## RESULT AND DISCUSSION

CoQ0 exhibits strong toxicity against various cancer cell lines [6,8,13,38]. Owing to its potent anti-cancer properties, we investigated the cytotoxic and apoptotic effects and possible mechanisms of action of CoQ0 in human chronic myeloid leukemia K562 cells.

In the present study, the concentration of CoQ0 between 5-20 μM significantly reduced the cell proliferation in K562 leukemia cells ($p<0.0001$; Figure 1). The $IC_{50}$ value of CoQ0 for 24 h was

calculated as 9.89 µM in K562 cells. CoQ0 has also been reported to decrease the proliferation of various cancer cells [6,8,38]. It has been described that the caspase-3 activation causes fragmentation of PARP which is a key protein involved in cell apoptosis [39-42]. Caspase-3 activity remained unchanged with 10 and 12.5 µM CoQ0 treatment in K562 cells (Figure 2).

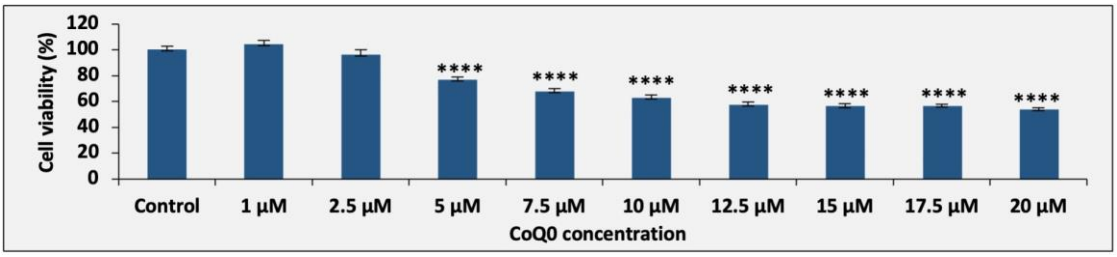


**Figure 1.** Effect of CoQ0 on the proliferation of K562 chronic leukemia cells $^{****}p<0.0001$ vs. control

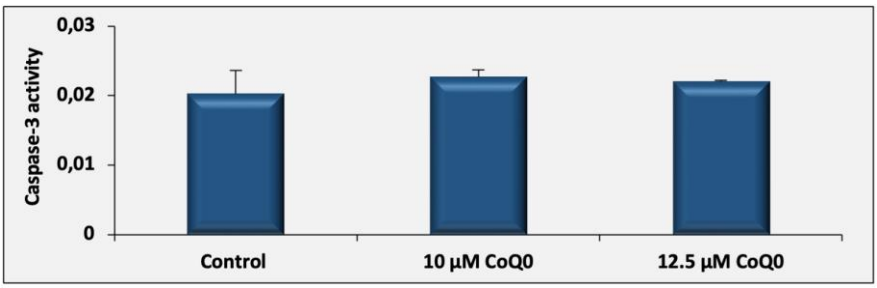


**Figure 2.** Effect of CoQ0 treatment on caspase-3 activity in K562 chronic leukemia cells

Maintaining a proper balance between the levels of anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) proteins is a crucial factor in preserving cellular homeostasis [43,44]. Bax protein expression demonstrated a decrease in 12.5 µM CoQ0 concentration (Figure 3A; $p<0.01$ vs control). Bcl-2 protein expression was decreased in CoQ0-treated groups as compared to the control group; however, this downregulation was not statistically significant at 10 and 12.5 µM CoQ0 concentrations (Figure 3A; p=0.0569, p=0,0666 respectively). Hseu et al. demonstrated a dose-dependent decrease of Bcl-2 and an accompanying increase in Bax expression by CoQ0 in various cancer cells [13,38]. Even if, high Bax expression is considered as a trigger of apoptosis, some apoptosis inducing agents have been shown to reduce Bax expression similar to our findings [45].

In particular, among the major groups of MAPKs, p44/42 (ERK1/2), and p38 MAPK signaling pathways are important targets in the cancer management and the activity of the MAPK is regulated by phosphorylation [21-23,46,47]. MAPK pathways have the potential to either facilitate or hinder the growth of cancerous cells depending on the cellular context [48]. Specifically, ERK may serve as an anti-apoptotic molecule that transduces survival signals, while p38 activation is associated with the induction of apoptosis [49-51]. Interestingly, p38 has been shown to mediate antiapoptotic/pro-growth signals in different systems [52-54]. Furthermore, ERK is a crucial component of the Ras/Raf/MEK/ERK signaling pathway that is regulated by Raf [55]. It has been reported that phosphorylation of the member of the Raf family, c-Raf at Ser259 prevents the activation of c-Raf and dephosphorylation of c-Raf at Ser259 is a pivotal part of the process of c-Raf activation [56]. In K562 cell line, the protein expression of p-c-Raf (Ser259) was decreased by 12.5 µM CoQ0 treatment (Figure 3B). Studies have demonstrated that Akt modulates the Erk pathway through c-Raf phosphorylation at Ser259, which is an inhibitory site [57]. The current investigation has revealed that CoQ0-induced deactivation of Akt leads to c-Raf activity enhancement by means of dephosphorylation in the K562 cell line. The results showed that CoQ0 at concentration of 10 µM also induced expression of p-p38 MAPK (Figure 3B). Moreover, treatment of K562 cells with 12.5 µM CoQ0 resulted in decreased phosphorylation of ERK1/2 which was not statistically significant (Figure 3B). Wang et al.

demonstrated that the induction of apoptosis in human chronic leukemia K562 cells by 2-hydroxy-3-methylanthraquinone is mediated through activation of p-p38 MAPK and downregulation of p-ERK1/2 [59]. In addition, different phosphorylation states of p38 MAPK can be seen in apoptotic cell death, whereas decreased p-p38 MAPK protein expression was shown in the induction of apoptosis in REH leukemia cells [60].

The Akt signaling pathway continues to be an essential pathway of interest for treatment of leukemia [32]. The phosphorylation of Akt is routinely used as a marker of Akt activation [50]. In the present findings, CoQ0 treatment (12.5 µM) decreased p-Akt (Ser473) protein expression, but the phosphorylation of Akt (Thr308) was not affected by treatment (Figure 3C).

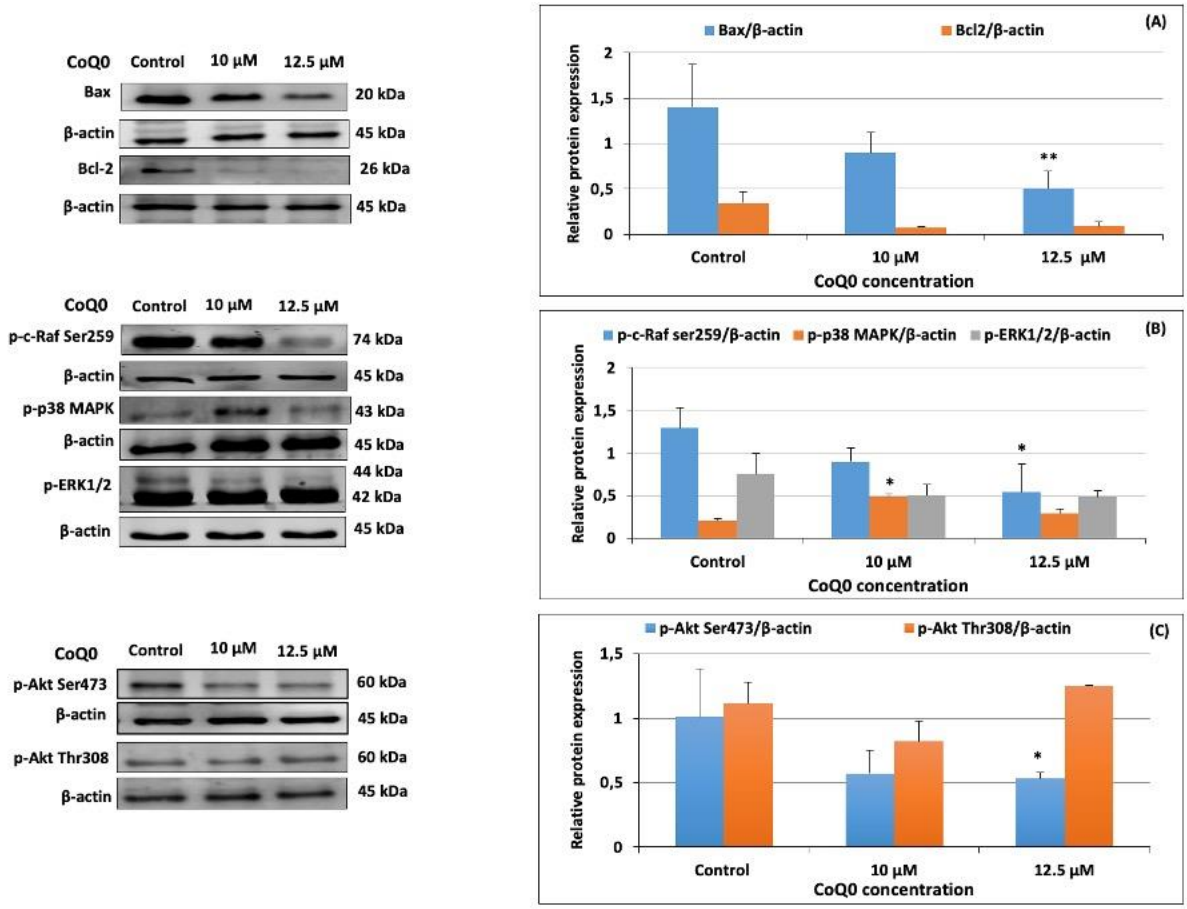


**Figure 3.** The effects of CoQ0 on the protein expression of apoptosis, MAPK signaling and AKT signaling-related proteins in K562 cells $^{*}p<0.05$ and $^{**}p<0.01$ vs. control

In accordance with these results, we can suggest that CoQ0 may inhibit Akt pathway by reducing Akt phosphorylation at Ser473 at the indicated doses. However, it must be noted that CoQ0 exhibited biphasic effects on p-p38 MAPK in K562 cells.

The results showed that CoQO demonstrated a significant cytotoxicity in chronic myeloid leukemia K562 cells. The antileukemia activity of CoQ0 in K562 cells could be related to the inhibition of phosphorylation of c-Raf at Ser259 and Akt at Ser473. In conclusion, these results contribute to understanding the anticancer activity of CoQ0 in chronic myeloid leukemia K562 cell line. Further studies are required to assess a promising potential of CoQ0 as an anticancer agent in chronic myeloid leukemia treatment.

## AUTHOR CONTRIBUTIONS

Concept: A.Z.K.; Design: A.Z.K.; Control: E.K.S., A.Y., A.Z.K.; Sources: A.Z.K.; Materials: E.K.S., A.Y., A.Z.K.; Data Collection and/or Processing: E.K.S., A.Y., A.Z.K.; Analysis and/or Interpretation: E.K.S., A.Y., A.Z.K.; Literature Review: E.K.S., A.Y., A.Z.K.; Manuscript Writing: E.K.S., A.Z.K.; Critical Review: E.K.S., A.Y., A.Z.K.; Other: -

## CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

## ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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