Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 8 Issue 1 January 2024

Research Article

Evaluating the Therapeutic Potential of Coenzyme Q10 in Colon Cancer

Aiman Alsaegh[1]*, Amirah Qadiri[2], Ghaida Nojoom[3], Alaa Banjar[4] and Ramya Sindi[1,5]

[1]Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences Umm Al-Qura University, Makkah, Saudi Arabia

[2]Histopathology Department, Dr. Suliman Alhabib Hospital, Jeddah, Saudi Arabia

[3]Pathology Department, High Quality Lab, Makkah, Saudi Arabia

[4]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

[5]Hekma School of Education, Health and Behavioural Sciences, Dar Al-Hekma University, Jeddah, Saudi Arabia

***Corresponding Author:** Aiman Alsaegh, Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences Umm Al-Qura University, Makkah, Saudi Arabia.

DOI: 10.31080/ASMS.2024.08.1739

Received: December 22, 2023

Published: December 28 , 2023

Abstract

Colorectal cancer (CRC) is the third most common malignancy around the world. Chemotherapy, particularly 5- Fluorouracil (5-FU), is the current first-line treatment strategy for CRC. However, chemoresistance restricted the therapeutic effectiveness of 5-FU, necessitating the exploration of novel treatment protocols. A growing body of evidence supported the efficacy of coenzyme Q10 (CoQ10) as a therapeutical option for pathological disorders. The study aimed to evaluate the potential therapeutic effect of CoQ10 as single or combined with 5-FU against CRC. The HCT116 cell lines were treated with monotherapy of CoQ10, 5-FU, and combined therapy for 48 hours, prior to the cell cycle and gene expression analysis. CoQ10 monotherapy arrested the cell cycle at the Sub-G1 phase and significantly increased the expression of p27 (3-fold) and Casp-3 (2.5-fold) while downregulating survivin (2-fold) and PCNA (5.5-fold) gene activity. Marked elevation in cell counts in the S and G2/M phase was observed following both 5-FU and concomitant cotherapy (CoQ10/5-FU). A comparable effect between 5-FU and combined therapy (CoQ10/5-FU) was also observed on the expression of cell cycle regulatory molecules, cell proliferation, and apoptosis markers. CoQ10 monotherapy showed a superior therapeutic effect to 5-FU and dual therapy (CoQ10/5-FU) highlighting it as a promising complementary/alternative candidate in CRC treatment possibly through its phenolic antioxidant activity and its role in mitochondrial respiration.

Keywords: Colorectal Cancer; Fetal Bovine Serum; World Health Organization

Abbreviations

5-FU: 5-Fluorouracil; CoQ10: Coenzyme Q10; CRC: Colorectal Carcinoma; DEME: Dulbecco's Modified Eagle Medium; FAP: Familiar Adenomatous Polyposis; FBS: Fetal Bovine Serum; FOBT: Fecal Occult Blood Test; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; PCR: Polymerase Chain Reaction; PI: Propidium Iodide; WHO: World Health Organization

Introduction

Colorectal cancer (CRC) is the third communal cancer around the world and the fourth reason for cancer mortality worldwide. The World Health Organization (WHO) estimates around 945000 new cases and 492,000 deaths annually [1]. CRC is characterized by aberrant increases in the levels of proteins that aid in cell cycle progression and cell survival, such as cyclin D1 (CCND1), CCND3, and B-cell lymphoma 2 (BCL2). Additionally, the disease is associated with decreased expression of cyclin-dependent kinase inhibitors (CDK; p21 and p27), Bcl-2-associated X protein (BAX), cytochrome C (Cyto-C), and caspase-3 (Casp-3) proteins. 5-Fluorouracil (5-FU) is the primary anti-cancer drug utilized to treat advanced colorectal cancer (CRC). It triggers cell death by increasing the expression of CDK-inhibitors and pro-apoptotic molecules. Nevertheless, malignant cells' resistance to 5-FU reduces their efficacy and survival rates. Hence, research efforts have been primarily dedicated to the development of more effective chemopreventive strategies [2].

Several nutritional supplements have emerged as potential substitutes or supplementary medications in the fight against cancer, particularly CRC [3,4]. Coenzyme Q10 (CoQ10) (also known as ubiquinone, ubiquinol, and ubidecarenone) is a vitamin-like substance found in tissues that require high energy or metabolic activity including heart, kidney, muscle, and liver [5]. The synthesis of CoQ10 within the body tends to decrease as one age and in cases of pathological conditions. Earlier reports have highlighted the association of CoQ10 deficiency with cancer disease progression [6]. Jolliet., *et al*. (1998) showed that CoQ10 levels were reduced in the plasma and the malignant tissue of breast cancer patients [7]. Moreover, later reports assessed the role of coenzyme Q10 supplementation on the serum level of interleukin 6 and interleukin 8 in breast cancer patients undertaking tamoxifen treatment. The study found that supplementation with CoQ10 was correlated with a significant reduction in interleukin-6 (IL-6) and interleukin-8 (IL-8) serum levels compared to the placebo group [8]. These studies highlight the potential role of CoQ10 supplementation in cancer therapy. Despite these findings, a limited number of studies have investigated the possible therapeutic role of CoQ10 in colon cancer. Hence, this study aimed to explore the effect of CoQ10 as a single and combined treatment with 5-FU on cell cycle progression and apoptosis of CRC cell line.

Materials and Methods

Cell culture and treatment

The human HCT116 colon cancer cell line was obtained from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle Medium (DMEM and RPMI-1640), foetal bovine serum (FBS), and antibiotic-antimycotic solution were supplied by Thermo Fisher Scientific (MT, USA). The HCT116 cells were maintained in DMEM supplemented with 10% FBS, and 1x antibiotic-antimycotic. All cells were incubated at 37 °C in a 5% CO2 atmosphere.

Coenzyme Q10 (CoQ10; 99.59% purity) was purchased from MedChemExpress LLC (NJ, USA). 5-FU was from Hospira Australia Ltd. (95% purity; Melbourne, Australia). The IC50 concentration for CoQ10 (49 µM) and 5-FU (50 µM) was obtained using the 3-(4,5Dimethylthiazol-2-yl)- 2,5-Diphenyltetrazolium Bromide (MTT) cytotoxicity test at 24 h (data not displayed). Cultured HCT116 cells were seeded in 6-well plates at a density of 1 × 10^5 cells/ml in a total volume of 2 ml/well and allowed to grow overnight. Following seeding, the cells were treated with 49 µM CoQ10, in the presence or absence of 50 µM 5-FU for 48 h. Negative control (NC) was prepared using Dimethyl Sulfoxide (DMSO). No toxicity was observed at the dilution of the maximum concentration of DMSO.

Cell cycle analysis

Following treatment with both COQ10 and combined therapy, the cells were washed, trypsinized, and subsequently handled according to the previously published method. In summary, 5 ml of PBS was used to wash the cells and thereafter the cells were treated with 0.5 ml of cold 70% ethanol for 24 hours at a temperature of 4 °C. Subsequently, cells were washed with PBS twice, then treated with 10 µM RNase A for a duration of 15 minutes, and finally stained with 2 µg/ml propidium iodide DNA dye. The cells stained with DNA were subsequently examined for their cell cycle phase using the Novocyte 3000 flow cytometer (Acea Biosciences Inc.; CA, USA). The cell cycle study was conducted utilizing the cell cycle algorithm provided by the NovoExpress program. The data presented indicates the percentage of each cell cycle stage: sub-G1, G0/G1, S, or G2/M (n = 3).

RNA extraction and cDNA synthesis

The extraction of total RNA from HCT116 cell line was conducted using the Purelink RNA mini kit (Thermo Fisher Scientific), according to the manufacturer's protocol. The isolated RNA was quantified using Nanodrop (BioSpecnano; Shimadzu Corporation, Kyoto, Japan). Next, cDNA was synthesized using 200 ng of RNA based on the manufacturer's instructions of a high-capacity RNA-to-cDNA reverse transcription kit (Thermo Fisher Scientific).

Quantitative Real Time-PCR the expression of six target genes (CCND1, p21, p27, Casp-3, PCNA and Survivin) was evaluated using the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). PCR was carried out in a total volume of 20 µL each harboring 7 µL DNase/RNase free water, 10 µL SYBR Green mix, 1 µL (5pmol) of each forward and reverse primer, together with 1 µL (25ng) of the synthesized cDNA. Negative control was included in each reaction where nuclease-free water was used as a template. Amplification was achieved using 40 cycles under the following conditions: 15 seconds at 95°C, and 1 minute at 65°C. The reaction was conducted in triplicate using SYBR green master mix from Applied Biosystems, (Thermo Fisher Scientific, Warrington, UK). Gene expression relative quantification was assessed using the $2^{-\Delta\Delta CT}$ method. The results were normalized against the expression of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Analysis outcomes were demonstrated as a fold increase or decrease when compared to the negative control group.

Statistical analysis

Statistical analysis was performed using SPSS version 25. The normality of each parameter was evaluated using the Kolmogorov-Smirnov test, and the homogeneity was verified using the Levene test. A one-way analysis of variance (ANOVA) was conducted, followed by either Tukey's honestly significant difference (HSD) or Games-Howell post-hoc tests, to assess the equality of variances between the groups. Pearson's test was used to determine correlations. A significance level of less than 0.05 was used to determine statistical significance.

Results

Effect of coenzyme Q10 and 5-FU drug on HCT116 cell cycle progression

Treatment protocol of HCT116 with coenzyme Q10 (CQ10) alone showed significant effect in increase cell numbers in the Sub-G1 phase of cell cycle (2-fold), whilst the percentages of cells in the other phases were reduced, relative to untreated control cells (Figure 1a). In contrast, 5-FU monotherapy significantly escalated the numbers of cells in the S (1.3-fold) and G2/M (2.5-fold) phases of cells cycle compared with non-treated and CQ10-treated cells as shown in (Figure 1c) and (Figure 1d). The combination thereby of CQ10 and 5-FU showed similar cell cycle effects to 5-FU single therapy and was associated with marked increases in the cell counts in S and G2/M phases, suggesting that the actions of 5-FU in the co-treatment group were dominant (Figure 1).

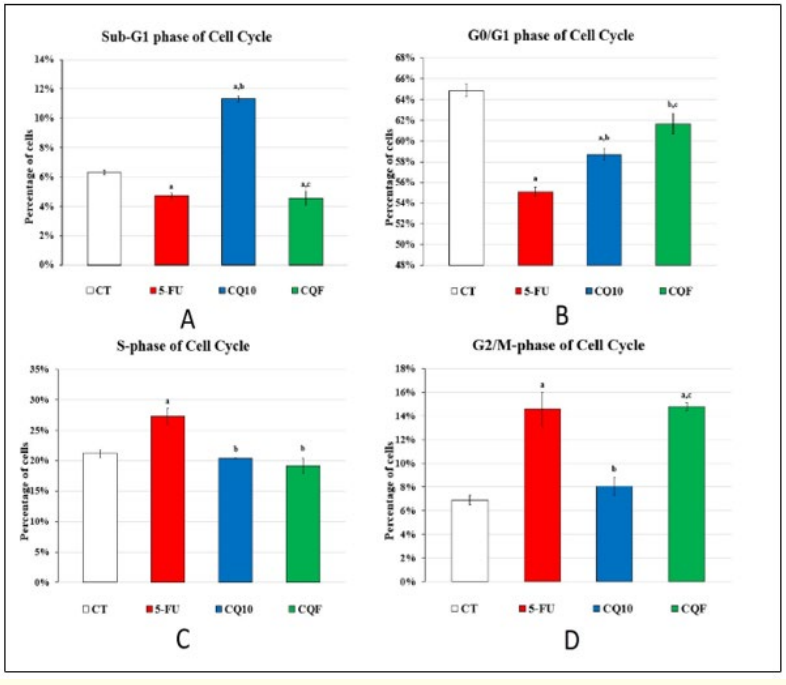

Figure 1: The effects of coenzyme Q10 and 5-FU drug on HCT116 cell cycle progression.

Percentage of cells (mean ± SD) in the various phases of the cell cycle in non-treated control cells, and following treatments with 5-Fluorouracil, Coenzyme Q10 single (5-FU & CoQ10), and combined (CQF) therapies for 48 h in the HCT116 colon cancer cell lines (a = $P < 0.05$ compared with CT, b = $P < 0.05$ compared with 5-FU, c = $P < 0.05$ compared with CoQ10)

Relative gene expression of cell cycle regulatory molecules

The expression of the cell cycle inducer gene, CCND1, was significantly reduced with 5-FU single treatment (5-fold) and coincided with a marked increase in the p21 (4-fold) cell cycle inhibitory genes in the HCT116 compared with untreated cells (Figure 2a). Contrariwise, CQ10 monotherapy showed minimal effects of the CCND1 and p21 genes, whilst significantly increased the expression of p27 (3-fold), relative to control and 5-FU-treated HCT116 cells. Additionally, the dual therapy group showed a similar, but weaker, expression profile to the 5-FU single therapy group in the HCT116 cells.

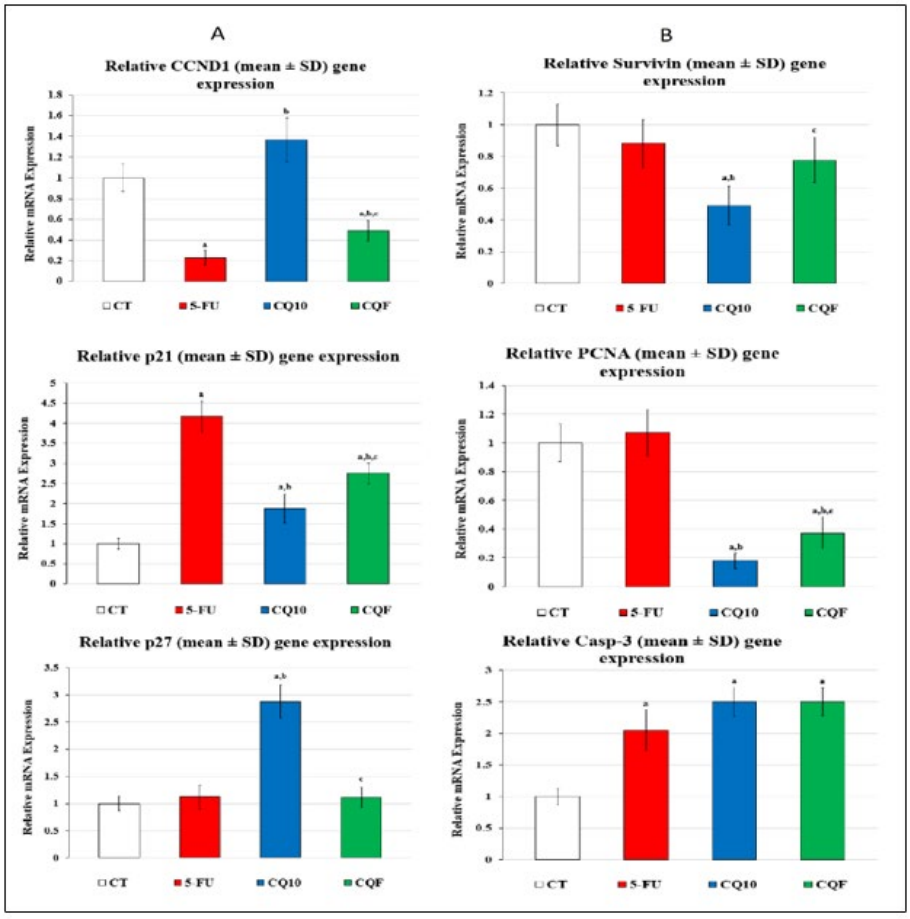

Figure 2: Describe the expression of genes that control cell cycle regulatory or proliferation or apoptosis in treated cell line HCT116, Real-Time PCR was used to determine the expression of genes (CCND1, p21, p27, Casp-3, PCNA, Survivin) (a = $P < 0.05$ compared with CT, b = $P < 0.05$ compared with 5-FU, c = $P < 0.05$ compared with CoQ10).

Relative gene expression of cell proliferation and apoptosis regulatory molecules

While the gene expression of survivin and PCNA cell proliferation markers were comparable between the 5-FU single treatment and untreated HCT116 cells, both genes were significantly (2fold and 5.5-fold, respectively) reduced in the CQ10 single therapy group (Figure 2b). Moreover, the mRNA expression of the pro-apoptotic molecule, Casp-3, was significantly increased with 5-FU and CQ10 single therapies (2.5-fold for both treatments) compared with the control cells (Figure 2b). However, no additional effect on the

expression of the targeted genes was observed in the dual therapy group relative to both single therapy groups (Figure 2b).

Discussion

This report evaluated the chemotherapeutic impact of single and combined treatment of CoQ10 and 5-FU against CRC in HCT116. The first-line therapy for CRC, 5-FU, is known to inhibit thymidylate synthase and trigger cell cycle arrest at S-phase [9,10]. In an earlier study, 5-FU was also associated with cell cycle arrest at the G2/M phase in HCT116 cell lines [11]. 5-FU has an evident role in suppressing tumor cell growth by regulating crucial pathways that promote cell death [12]. The 5-FU treatment induces prooxidant conditions by generating reactive oxygen species (ROS) that damage DNA and ultimately lead to cell apoptosis [13]. However, the development of drug resistance to chemotherapy severely restricted the clinical utility of 5-FU. Several mechanisms underlie the observed chemoresistance by tumor cells, including the antioxidant defense mechanism [13]. Hence, the phenolic antioxidant activity of CoQ10 contributes significantly to cellular resistance to chemotherapy. Notably, CoQ10 also demonstrates pro-oxidant behavior which can arise from either a semiquinone (free radical derived from quinones) interaction with CoQ10 or because of the reaction of oxygen with the oxidized form of CoQ10 [14].

The study found that treatment with 5-FU alone caused a halt in the cell cycle during the S-phase, while also boosting the amount of cells in the G2/M phase. The expression of cell proliferative markers (CCND1) was significantly reduced following 5-FU single therapy, while it also elevated the transcriptional activity of cell cycle inhibitory (p21) and pro-apoptotic (Casp-3) molecules. However, a limited effect was witnessed in the expression of survivin, PCNA, and p27 genes. This observation aligns with previous reports highlighting the association of 5-FU monotherapy with cell cycle arrest and apoptosis [15-18]. However, the efficacy of 5-FU in advanced stages of tumor is limited because of the frequent development of resistance by malignant cells to this cytotoxic drug [12,13]. It is for this reason that there is an urgent requirement for the development of more effective therapeutic techniques against colon cancer.

In this report, CoQ10 monotherapy was associated with increased cell accumulation in the SuBG1 phase together with upregulation of cell cycle inhibitory molecules (p21 and p21). In addition, CoQ10 single treatment was correlated with reducing survival markers expression. These data highlight the role of CoQ10 in inducing cell cycle arrest and tumor cell apoptosis. Our findings are in agreement with an earlier report in which Jinlian., *et al*. (2022) demonstrated the inhibitory effect of decylubiquinone (CoQ10 analog) on colorectal cancer growth and metastasis by suppressing cell proliferation and upregulating Sirtuin2 (SIRT2) in CT26 mice model and patientderived xenograft [19]. Furthermore, CoQ10 showed promising anti-cancer effects against pancreatic cancer by targeting mitochondrial function. Dadali., *et al*. (2021) highlighted the association of oxidized CoQ10 (ubidecarenone) with increased ROS production and the activation of apoptosis in pancreatic cancer models [20]. Moreover, Coenzyme Q10 functions as an electron and proton transporter in the mitochondrial respiratory chain and has the potential to disrupt mitochondrial activity, thereby impacting tumor metabolism [14]. Therefore, Coenzyme Q10 may inhibit CRC growth by affecting the metabolism of colorectal cancer.

The present study further evaluated the effect of dual therapy of CoQ10 and 5-FU on colon cancer cells. The combined regimen resulted in significant increases in cell counts during the S and G2/M phases, which was comparable to the effects of 5-FU alone therapy. The co-therapy protocol also revealed a similar impact to 5-FU on the functional activity of cell cycle regulatory molecules. Additionally, A limited effect on mRNA expression of cell proliferation markers was observed following the combined treatment when compared to the monotherapy of CoQ10 or 5-FU. A plausible explanation of the observed result is the prospective role of CoQ10 in reducing the cytotoxic effect of chemotherapy. A considerable body of work has been devoted to investigating the potential of supplemental antioxidants administered during chemotherapy to safeguard healthy tissue while preserving tumor control. A recent *in vivo* report revealed that CoQ10 supplements effectively mitigated the impact of 5-FU-induced release of free radicals on mice cells, thus restoring them to their normal state [21]. Moreover, the combination treatment of anthracyclines (ANT) and CoQ10 was associated with improved cardiac function in two cohorts of children diagnosed with leukemia and non-Hodgkin lymphoma [22]. However, additional experimental analysis is required to understand the mechanism by which CoQ10 reduces the toxic effect of chemotherapy.

The observation of this report is limited to the HCT116 cell line. Thus, further work could determine the effectiveness of coenzyme Q10 as a therapeutic agent for different stages of CRC using other cell lines and *in vivo* models. Additionally, the present study adopted a concomitant combination regimen, and subsequent analyses that utilize consecutive dual therapy protocol (5FU followed by CoQ10 or vice versa) are required to accurately determine the optimal therapeutic strategy for CRC. Moreover, future *in vitro* analysis could evaluate the tumoricidal effect of dual and monotherapy of CoQ10 and 5-FU related to cell cycle and apoptosis at several time points (e.g., 12, 24 and 72h). An extension of this work should evaluate the expression of glycolysis markers and oxidative metabolism-related enzymes to support the current observations.

In conclusion, the current data suggests that CoQ10 yields a superior therapeutic effect than both 5-FU and the CoQ10/5-FU dual therapy. The single regimen of CoQ10 arrested the cell cycle at the Sub-G1 and increased the expression of Casp-3 and p27 while downregulating survivin and PCNA gene activity. To the best of our understanding, this is the first study to investigate the use of CoQ10 and 5-FU as combined therapy for CRC. However, concomitant co-therapy showed a comparable impact to 5-FU. Thus, further analysis is required to precisely assess the anti-cancer effect of CoQ10 and 5-FU sequentially and concurrently at multiple time points, while evaluating their impact on the expression of oxidative phosphorylation markers.

Bibliography

1. Stewart BW., *et al*. "International Agency for Research on, World cancer report. 2014, Lyon: International Agency for Research on Cancer. 630 pages: illustrations (black and white, and colour).
2. Xie YH., *et al*. "Comprehensive review of targeted therapy for colorectal cancer". *Signal Transduction and Targeted Therapy* 5.1 (2020): 22.
3. Huang XM., *et al*. "Natural products for treating colorectal cancer: A mechanistic review". *Biomed Pharmacotherapy* 117 (2019): 109142.
4. Rejhova A., *et al*. "Natural compounds and combination therapy in colorectal cancer treatment". *European Journal of Medicinal Chemistry* 144 (2018): 582-594.
5. Singh U., *et al*. "Coenzyme Q10 supplementation and heart failure". *Nutrition Review* 65.6 (2007): 286-293.
6. Garrido-Maraver J., *et al*. "Clinical applications of coenzyme Q10". *Frontiers in Bioscience (Landmark Ed),* 19.4 (2014): 619-633.
7. Jolliet P., *et al*. "Plasma coenzyme Q10 concentrations in breast cancer: prognosis and therapeutic consequences". *International Journal of Clinical Pharmacology and Therapeutics* 36.9 (1998): 506-509.
8. Zahrooni N., *et al*. "The Effect of Coenzyme Q10 Supplementation on Vascular Endothelial Growth Factor and Serum Levels of Interleukin 6 and 8 in Women with Breast Cancer: A Double-Blind, Placebo-Controlled, Randomized Clinical Trial". *Therapeutics and Clinical Risk Management* 15 (2019): 1403-1410.
9. Can G., *et al*. "5-Fluorouracil signaling through a calcium-calmodulin-dependent pathway is required for p53 activation and apoptosis in colon carcinoma cells". *Oncogene* 32.38 (2013): 4529-4538.
10. Akpinar B., *et al*. "Correction: 5-Fluorouracil-induced RNA stress engages a TRAIL-DISCdependent apoptosis axis facilitated by p53". *Oncotarget* 7.44 (2016): 72380.
11. Yoshikawa R., *et al*. "Dual antitumor effects of 5-fluorouracil on the cell cycle in colorectal carcinoma cells: a novel target mechanism concept for pharmacokinetic modulating chemotherapy". *Cancer Research* 61.3 (2001): 1029-1037.
12. Kim EJ., *et al*. "Over-activation of AKT signaling leading to 5-Fluorouracil resistance in SNU-C5/5-FU cells". *Oncotarget* 9.28 (2018): 19911-19928.
13. Blondy S., *et al*. "5-Fluorouracil resistance mechanisms in colorectal cancer: From classical pathways to promising processes". *Cancer Science* 111.9 (2020): 3142-3154.
14. Treberg JR., *et al*. "Evidence for two sites of superoxide production by mitochondrial NADH-ubiquinone oxidoreductase (complex I)". *Journal of Biological Chemistry* 286.31 (2011): 27103-27110.
15. Oh HJ., *et al*. "p53 expression status is associated with cancer-specific survival in stage III and high-risk stage II colorectal cancer patients treated with oxaliplatin-based adjuvant chemotherapy". *British Journal of Cancer* 120.8 (2019): 797-805.

16. Basak D., *et al*. "The Role of Oxidative Stress and Its Counteractive Utility in Colorectal Cancer (CRC)". *Cancers (Basel)* 12.11 (2020).

17. Liu Q., *et al*. "Modulating p-AMPK/mTOR Pathway of Mitochondrial Dysfunction Caused by MTERF1 Abnormal Expression in Colorectal Cancer Cells". *International Journal of Molecular Sciences* 23.20 (2022).

18. Mihanfar A., *et al*. "Melatonin increases 5-flurouracil-mediated apoptosis of colorectal cancer cells through enhancing oxidative stress and downregulating survivin and XIAP". *Bioimpacts* 11.4 (2021): 253-261.

19. Li, J., *et al*. "Decylubiquinone Inhibits Colorectal Cancer Growth Through Upregulating Sirtuin2". *Frontiers in Pharmacology* 12 (2021): 804265.

20. Dadali T., *et al*. "Elevated levels of mitochondrial CoQ (10) induce ROS-mediated apoptosis in pancreatic cancer". *Scientific Report* 11.1 (2021): 5749.

21. Oluwatosin AA. "The Effect of Dietary Co-enzyme Q10 Supplementation on Haematological Parameters and Splenic Morphology in Mouse Model of 5-FluorouracilInduced Toxicity". *Journal of Biomedical and Pharmaceutical Sciences* 5.11 (2022): 1-19.

22. Iarussi D., *et al*. "Protective effect of coenzyme Q10 on anthracyclines cardiotoxicity: control study in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma". *Molecular Aspects of Medicine* 15 (1994): s207-212.