# Effect of Quercetin on Tumor-Derived Exosomal miRNA Circulation in Primary (Colo 320) and Metastatic (Colo 741) Colon Cancer Cell Lines

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# Abstract

**Objective**: Quercetin is a phytochemical that is regarded as a potential anticancer agent in colon cancer prevention. Exosomes are nanovesicles secreted by normal or cancer cells, which transport miRNAs and proteins and participate in intercellular communication as a cargo. Cytotoxicity, exosomal miRNA secretion, and distribution of Dicer, Ago2, CD9, CD63 and eIF2α in quercetin applied Colo 320 and Colo 741 colon cancer cell lines were aimed.

**Materials and Methods**: The cytotoxicity test that we used to evaluate cell viability is MTT assay. The distribution of Dicer, Ago2, CD9, CD63 and eIF2α in both cell lines were analyzed using the indirect immunoperoxidase technique. The exosomal miRNA levels were evaluated by using a miRCURY™ Kit.

**Results:** Decreased eIf2α immunoreactivity following quercetin application was significant in Colo 741 colon cancer cells. Increased Dicer and CD9 immunoreactivities were significant between Colo 320 and Colo 741 colon cancer cells. Additionally, after quercetin application, exosomal miRNA concentrations were increased in both Colo 320 and Colo 741 cell lines.

**Conclusion:** According to our results, total exosomal miRNA concentration and levels of associated proteins were affected after quercetin administration. Additionally, the change in the immunoreactivity of the related proteins after quercetin application differed according to the cell type and origin.


# Introduction

The World Health Organization reports that colorectal cancer is the third most prevalent cancer type. Also, the incidence varies between genders; it is the third most common cancer in men and the second in women [1]. CRC (colorectal cancer) formation arises from the development of genetic and epigenetic transformation in the colon epithelium cells [2]. Recently, it has been composed that the tumor microenvironment, which contains stromal cells, factors and different immune cell populations, is very important in the formation, invasion and metastasis of colon cancer cells. Furthermore, the tumor microenvironment regulates signaling pathways and transcription factors, which are known to play a substantial role in the initiation, metastasis and invasion of colon cancer cells [3, 4].

Exosomes are membrane-bound extracellular vesicles (30–200 nm) secreted by normal or cancer cells and contribute to intracellular communication [5, 6]. The exosomes composition varies according to cell type, but they are important parts of the tumor microenvironment. Exosomes contain characteristic tetramer membrane proteins such as CD9, CD63 and CD151 on their surface and participate in intercellular communication by transporting proteins, miRNAs, mRNAs, lncRNAs and DNA fragments [7]. Many studies have reported that exosomal miRNAs and proteins may actively regulate tumorigenesis by inhibiting immune surveillance as well as by increasing chemoresistance in colon cancer [8, 9]. Also, the exosomal miRNAs have potency toward use as biomarkers for early diagnosis and prognosis assessment in colon cancer [10, 11].

miRNAs are small non-coding RNAs that participate in a variety of functions such as proliferation, migration, apoptosis, and epithelial-mesenchymal transformation in cancer cells [12]. The miRNAs biogenesis begins within the nucleus with the primary hairpin miRNA (pri- miRNA) transcript polymerization by RNA polymerase- II enzyme. Then, the microprocessor complex (Drosha– DGCR8) cleavages and digests the pri- miRNA to produce the pre- miRNA. The exportin 5/RanGTP complex transports pre- miRs from the nucleus to the cytoplasm. In the cytosol, Dicer, TRBP and Paz proteins cleave the pre- miRNA hairpin and mature duplex miRNA is produced. Mature miRNAs form RISC complex with argonaute protein 2 (Ago2) or they can be loaded to exosomes and then released [13]. Current studies have reported that Ago2, Dicer, and exosomal miRNAs expression levels are correlated with patients' clinical outcomes and overall survival in colon cancer [14, 15, 16, 17]. Moreover, an elevated level of Dicer mRNA expression in mucosa cells is significantly related with poor survival in colorectal cancer patients [18]. These results indicate that miRNAs and miRNA biogenesis factors are crucial post-transcriptional modulators that have critical roles in health and disease. Therefore, miRNAs and their biogenesis factors, including Drosha, Ago2 and Dicer, are being largely studied in relation to target cancer treatment [19, 20].

In colorectal cancer treatment, multi aims and diverse action mechanisms with reduced toxicity yield improved clinical outcomes. In the last decade, the synergistic effects of polyphenols with chemotherapeutic drugs have been investigated. Dietary polyphenols affect disparate cellular processes by acting as chemopreventive blockers in colorectal cancer [21]. Quercetin is one of the most commonly investigated polyphenolic compounds with regard to its anticancer activity against colorectal cancer. Studies show that depending on the dosage and incubation time, quercetin is effective in carcinogenesis and apoptosis by affecting the cell viability, level of apoptotic proteins, and cell cycle [22, 23]. Additionally, it is thought that quercetin may be effective in cancer development, metastasis, and chemo-resistance by affecting tetraspanins and tumor derived exosomal miRNAs, which are effective in cell to cell interaction, cell adhesion, migration, proliferation, and metastasis in colorectal cancer [19, 20].

In latest years, studies on the search for individualized treatment methods with fewer side effects according to the cancer cell origin and the type of cancer have gained importance. Studies have shown that polyphenol quercetin has the potential to be a new alternative agent in cancer treatment due to its strong anti-oxidant and anti-cancer effects. However, the effects of quercetin on exosome synthesis and exosomal miRNA secretion, which are effective in the differentiation, proliferation, migration and metastasis of cancer cells, have not been examined in concert in both Colo 320 and Colo 741 colorectal cell lines. Furthermore, eukaryotic translation initiation factor 2α (eIF2α) is a factor that plays a crucial role in protein synthesis regulation. The eIF2α dysregulation is associated with certain pathologies, including metabolic disorders and cancer [24]. To the best of our knowledge, not any studies have addressed the effect of quercetin on eIF2α expression in colon cancer with in vitro and in vivo studies. In this study, the aim was to research the effects of quercetin on carcinogenesis according to its effects on the exosomal miRNA level and CD9, CD63, Dicer, Ago2, and eIF2α expression in Colo 320 and metastatic Colo 741 colon cancer cell lines.

## Methodology

# Cell Lines and Cell Culture

In this study, two cell lines were used; Colo 320 (ATCC: CCL-220.1) and Colo 741 (ECACC: 93052621) colon cancer cell lines. The cell lines were cultured in RPMI 1640 medium (Biochrom; FG- 1215) with 10% Fetal Bovine Serum ((FBS) Capricorn Scientific; FBS- 11B), 1% penicillin-streptomycin (Biochrom; A- 2213) and 1% L- glutamine (EMD Millipore; K- 0282). The cell cultures were maintained in a moisturized atmosphere of 5% CO at 37˚C.

# Cell Viability Assay

The cell viability analysis of quercetin was studied using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 Diphenyltetrazolium Bromide) (Biotium, #30006) assay. The MTT experiments were performed according to our previous report with slight modifications [25]. The two cancer cell lines were applied with different quercetin (2- (3, 4- dihydroxyphenyl)- 3, 5, 7- trihydroxy- 4H- 1- benzopyran- 4- one, 3, 30, 40, 5, 6 pentahydroxyflavone; 95% pure) (Sigma; Q- 4951) concentrations (100, 50, 25, 10, 5 µg/ml) and then incubated for 24h or 48 h at 37˚C. The effective dose and incubation time were determined as 25 µg/ml quercetin administration for 48 hours. Immunocytochemical analyses were performed in four groups. The Colo 320 and Colo 741 control groups, which were cultured with standard culture medium and the other study groups were 25 µg/ml quercetin applied Colo 320 and Colo 741 cells. The experiments were repeated three times.

# Immunocytochemistry (Ihc)

The previously described indirect immunoperoxidase staining protocols were used for evaluating Dicer, eIF2α, eIF2c (Ago2), CD9, and CD63 distributions in Colo 320 and Colo 741 cells [25]. Briefly, 5000 cells were plated in a 24- well plate and incubated overnight and, then were applied with quercetin. The cells were fixed with 4% paraformaldehyde after incubation. After washing with phosphate buffer saline (PBS), they were incubated with inhibiting solution for 1 h, cells were then incubated with primary antibodies; anti- Dicer (Santa Cruz sc- 136981), anti- eIF2α (Santa Cruz sc- 133132), anti- Ago2 (Santa Cruz sc- 376692), anti- CD9 (Santa Cruz sc- 13118) and anti- CD63 (Santa Cruz sc- 5275) overnight at 4°C. After washing with PBS, cells were subsequently incubated with biotinylated secondary antibody (Histo Stain-Plus, HRP, 859043, Thermo Fischer) and streptavidin-peroxidase complex, respectively. For immune labeling, cells were incubated with diaminobenzidine ((DAB) Scytec; 36038) for five minutes after washing with PBS. Then, cells were counterstained with Mayer's hematoxylin solution (Bio Optica; 1213) for five minutes. They were mounted using the mounting medium (Merck Millipore- 107961- Germany) and examined under a light microscope (Olympus- BX 40, Tokyo, Japan).

H-SCORE was used for semi-quantitative grading of Dicer, eIF2α, Ago2, CD9, and CD63 staining. In the H-SCORE = Σл (i + 1) formula (i: insistence of dyeing with a value of 1, 2 or 3 (mild, moderate, or strong, respectively), л: percentage of cells stained (between 0 and 100%)).

# Exosomal Mirna Analysis

The culture media were also collected for evaluation of exosomal miRNA levels from both control and quercetin application groups from Colo 320 and Colo 741 cells at 48 h. A miRCURY™ (Exiqon 300102) RNA isolation kit was used according to the manufacturer's guidelines.

# Statistical Analysis

Mean ± standard deviation was used for data expression. Graph Pad Prism 7 software was used for analysis and differences between groups were examined with Mann- Whitney U tests. A $p < 0.05$ was respected statistically significant.

## Results

# Cell Viability and Cytotoxicity

Five different concentrations (100, 50, 25, 10, 5 µg/ml) and two different incubation times (24 and 48 hours) were applied to Colo 320 and Colo 741 colon cancer cell lines to determine the effective dose and incubation time to be used in experiments. The effective concentration for the inhibition of cell growth in all two cell lines was established to be 25 µg/ml quercetin for 48 hours (Fig. 1).

# Immunohistochemical Assessment

The immunostaining intensity of Dicer was weak in quercetin applied Colo 320 primary colorectal cancer cells (Table 1). Additionally, the staining intensity of Dicer was increased after quercetin application in Colo 320 cell line, but the difference was not statistically significant when compared with Colo 320 control group (Fig. 2A- 2B, Table 2). The immunostaining of elf2α was strong in quercetin applied Colo 320 cells in comparison with a control group (Table 1). Otherwise, immunoreactivity of elf2α was decreased in quercetin applied Colo 320 cell line but the decrease was not statistically significant (Fig. 2C- 2D, Table 2). Immunostaining of Ago2 was weak in both the control group and quercetin applied Colo 320 cells (Table 1). Also, the staining intensity of Ago2 was decreased in quercetin applied Colo 320 cells but the difference was not statistically significant when compared with the control group (Fig. 2E- 2F, Table 2). Immunostaining of CD9 was weak in quercetin applied Colo 320 cell line (Table 1). The staining intensity of CD9 was increased in quercetin applied Colo 320 cells in comparison to the control group; however, the increase was not statistically significant ($p > 0,05$, Fig. 2G- 2H, Table 2). The immunostaining intensity of CD63 was moderate in quercetin applied Colo 320 cell line. Although immunoreactivity was

increased after 25 µg/ml quercetin application for 48 hours, the increase was not statistically significant when compared with the control group (Fig. 2I- 2J, Table 2).

Table 1
The intensity of Dicer, eIf2α, Ago2, CD9 and CD63 immunolabelling in Colo 320 and Colo 741 cells were applied with 25µg/ml quercetin for 48 h.

| | Colo-320 cells | | Colo-741 cells | |
|---|---|---|---|---|
| | Control group | Quercetin | Control group | Quercetin |
| Dicer | - | +/- | + + + | + + + |
| elf2α | + + | + + + | + + + + | + + + + |
| Ago2 | -/+ | -/+ | -/+ | -/+ |
| CD9 | -/+ | +/- | -/+ | + + |
| CD63 | + | + + | -/+ | +/- |

Table 2
The H-SCORE of Dicer, eIf2α, Ago2, CD9 and CD63 immunolabelling in Colo 320 and Colo 741 cells applied with 25µg/ml quercetin for 48 h.

| | Colo-320 cells | | Colo-741 cells | |
|---|---|---|---|---|
| | Control group | Quercetin | Control group | Quercetin |
| Dicer | 121,3 ± 15 | 171,4 ± 40,68** | 277,5 ± 33,29 | 325 ± 50 |
| elf2α | 214,3 ± 38,39 | 211,2 ± 41,63 | 365 ± 19,15 | 275 ± 86,6* |
| Ago2 | 107,2 ± 6,974 | 102,5 ± 5 | 127,5 ± 32,02 | 110,4 ± 12,5 |
| CD9 | 107,5 ± 9,574 | 108,8 ± 4,72** | 112,5 ± 25 | 191,9 ± 21,38* |
| CD63 | 173,8 ± 19,67 | 197,7 ± 10,47** | 124,2 ± 48 | 115 ± 19,15 |

Data are expressed as means ± SD and were compared by Mann-Whitney U test.

* The data was significant when compared with control group ($p < 0.05$).

** The data was significant when compared with Colo-741 group ($p < 0.05$).

Immunostaining of Dicer was strong both in control and quercetin applied Colo 741 metastatic colorectal cancer cell line. Although Dicer staining intensity was increased after application of 25 µg/ml quercetin for 48 hours, this increase was not statistically significant (Fig. 3A- 3B, Table 2). The intensity of eIf2α was strong in Colo 741 metastatic colon cancer cell line (Table 1). In addition, immunoreactivity of eIf2α was significantly increased in quercetin applied to Colo 741 colorectal cancer cells compared to the control group (Fig. 3C- 3D, Table 2). The immunostaining of Ago2 was weak in both control and quercetin

applied metastatic colon cancer (Colo 741) cell line. Moreover, the staining intensity of Ago2 was decreased in quercetin applied Colo 741 cancer cell line ($p > 0,05$, Fig. 3E- 3F, Table 2). Immunostaining of CD9 was moderate in quercetin applied Colo 741 cell line (Table 1). Besides, the immunoreactivity of CD9 was increased after quercetin application, this increase was statistically significant in comparison with the control group ($p < 0,05$, Fig. 3G-3H, Table 2). The staining intensity of CD63 was weak in both control and quercetin applied Colo 741 cancer cell line (Table 1). The immunoreactivity of CD63 was decreased in quercetin applied Colo 741 metastatic colon cancer cell line but the difference was not statistically significant (Fig. 3I- 3J, Table 2).

# Exosomal Mirna Analysis

Exosomal miRNA isolation results indicate that miRNA concentrations increased in Colo 320 and Colo 741 colon adenocarcinoma cells culture media after administration of 25 µg/ml quercetin for 48 hours, but this increase has no statistical significance ($p > 0.05$, Table 3).

Table 3
Exosomal miRNA isolation results of Colo 320 and Colo 741 cells were applied with quercetin at a concentration of 25 µg / ml for 48 hours.

| | Control groups | Quercetin |
|---|---|---|
| **Colo-320 cells** | 11 ng/µl | 12,13 ng/µl |
| **Colo-741 cells** | 11,88 ng/µl | 15,15 ng/µl |

# Discussion

Colorectal cancer is one of the prevalent widespread forms of cancer globally. Acquired biological properties and functional capabilities of colon cancer cells have important hallmarks such as immortality, sustaining cell proliferative signaling, and stimulating angiogenesis and metastasis. Also, the tumor microenvironment is a fundamental factor in the initiation, progression, metastasis, and therapy response of colon cancer cells [26]. Additionally, the metastatic properties of colon carcinoma or metastasis of other types of cancer cells into the colon are other issues that need to be investigated [27].

According to CDC data, the 5- year survival percentage for colorectal cancer cases is 63.8%. Besides surgical intervention and radiotherapy, chemotherapy is the most common cancer treatment method [28]. However, chemoresistance and the side effects of chemotherapeutic drugs have led to the search for new agents with fewer side reactions in cancer treatment. Polyphenols are natural compounds that are used for chemoprevention and alternative approaches to decrease the mortality rate in CRC. Various researches have demonstrated that polyphenols can inhibit the overall carcinogenesis process by stimulating apoptosis through several mechanisms. Quercetin, which belongs to the natural polyphenols group, is the most studied flavonoid against CRC. Quercetin is commonly found in onion, apple,

strawberry, broccoli, tea, and red wine, which are frequently included in our diet [29]. In recent years, due to its effects on biosignalization and carcinogenesis, quercetin has been frequently used in anti-cancer research [30].

Recent experimental research have demonstrated that quercetin inhibits cancer cell proliferation and viability based on incubation time and dosage. *Rafiq et al.*, found that the application of B16F10 melanoma cell line with 5μM quercetin for 24 hours affected cell viability [31]. Also, *Zhang et al.*, found that the application of Caco- 2 and SW- 620 cell lines with 20 μM quercetin application for 24 hours had an effect on cell viability [32]. According to the study of *Han et al.*, Caco- 2 cell viability was inhibited as a result of 20 μM quercetin application for 24 hours [33]. On the basis of our previous report, the affective concentration was found to be 25 μg/ml for 48 hours in two of the cell lines Colo 320 and Colo 741 [23].

Exosomes are nano-sized vesicles and a crucial aspect of the tumor- microenvironment. Exosomes and exosom-derived miRNAs or proteins that are released by tumor cells contribute to the tumor micro-environment and may play an essential role in CRC progression. Therefore, there has been increased attention to cargo molecules that are found in exosomes as potential tumor markers and treatment targets for colorectal cancer [27]. They can transfer miRNAs, proteins, mRNAs, and DNA fragments from donor cells to distant recipient cells [4]. Recently, accumulating evidence shows that differentially expressed proteins and miRNAs in exosomes are latent novel biomarkers for CRC confirmation. Additionally, studies have demonstrated that different miRNAs and protein content in the blood exosomes of CRC patients may be beneficial prognostic predictors and would support the determination of innovative therapeutic strategies [34, 35]. Up to the present time, no study has investigated the effects of quercetin with respect to the expression of exosome marker proteins and miRNA biogenesis related proteins and exosomal miRNA levels in Colo 320 and Colo 741 tumor cells.

In our study, we reported that the immunoreactivity of CD9 has statistically significant increase in quercetin applied Colo 741 cells when compared to the control group. Also, the immunostaining intensity of CD9 was significantly higher in Colo 741 cells than Colo 320 cells after administration of quercetin. On the other hand, the immunoreactivity of CD63 was increased in Colo 320 primary colon cancer cell line and decreased in Colo 741 metastatic colon cancer line, after the application of quercetin. The immunostaining intensity of CD63 has statistical significant elevation in quercetin applied Colo 320 cells when compared with quercetin applied Colo 741 cells. It can therefore be finalized that Colo 320 and Colo 741 colon cancer cells secreted exosomes in response to quercetin administration and to use of exosomes for colon carcinoma identification, both CD9 and CD63 should be used because of diverse exosomal surface proteins secretion.

miRNAs are non-coding RNAs that regulate a large number of biological processes by inhibiting target gene expression. Functional studies have investigated that miRNAs have pivotal functional roles in CRC carcinogenesis and prognosis. Furthermore, emerging evidence indicates that miRNAs are robust biomarkers for the diagnosis and surveillance of CRC patients [12]. Current evidence indicates that miRNAs biogenesis and related proteins such as Dicer and Ago2 have effects on the development of

several cancers. *Chiosea et al.*, showed that pre-cancerous lesions and invasive lung adenocarcinomas were related to Dicer gene locus deletion [36]. The molecular mechanisms that regulate Dicer-related carcinogenesis in CRC are still recondite. *Faber* et al., showed that Dicer protein overexpression could serve as a poor prognosticator in CRC [17]. Nonetheless, previous studies have documented that Dicer mRNA levels were not significantly correlated with patient survival [14, 37]. On the other hand, *Iliou et al.*, reported that Dicer defects were linked to the colorectal cancer cells subpopulations generation with upregulated stem cell markers [38]. In light of these well-documented reports, we establish that the immunoreactivity of Dicer was increased in both quercetins applied Colo 320 and Colo 741 cells lines when compared with their control groups, but the elevations have no statistical significance. Moreover, the immunoreactivity of Dicer significantly increased in quercetin applied Colo 741 cells in comparison to quercetin applied Colo 320 cells. These data lead to the hypothesis that quercetin could have protective effects by the upregulation of tumor suppressive miRNA by increasing Dicer expression in colorectal cancer.

Ago2 is a key regulator of miRNAs in target mRNAs degradation through its catalytic activity in processes of gene silencing. The Ago2 protein has been found to be over-expressed in colon cancer, ovarian carcinoma, gastric carcinoma and colorectal carcinoma [39, 40, 41, 14]. Additionally, it has been shown that Ago2 over- expression was related to cancer aspects, including tumor cell proliferation, growth, and overall cancer patient survival [42]. However, the expression level of Dicer can be different from Ago2 expression level. It was found that the upregulated Dicer mRNA expression levels were not positively correlated with Ago2 mRNA expression levels [43]. Our results revealed that Ago2 immunoreactivity was decreased in quercetin applied Colo 320 and Colo 741 cells when compared with control groups, the decreases have not any statistical significance in both cell lines. Therefore, it may be regarded that the incubation time needs to be lengthened in order to detect a statistically significant decrease in Ago2 immunoreactivity after quercetin application in two cell lines Colo 320 and Colo 741.

Recent studies have reported that quercetin can elicit obvious endoplasmic reticulum stress in a variety of tumor cells [44, 45]. The different type of cellular stress leads to translation downregulation in cells, which is thought to save energy and encourage survival. The best-characterized downregulation mechanism for the translation regulation is the phosphorylation of eukaryotic initiation factor eIF2α in eukaryotic cells. Also, translational attenuation can stimulate apoptosis and autophagy in cells [46]. According to our experimental results, the staining intensity of eIf2α was decreased in all two Colo 320 and Colo 741 colon cancer cell lines after quercetin application; however, the decrease was only significant in the Colo 741 colon cancer cell line. In our very recent publication, we found that quercetin triggered apoptosis in Colo 320 and Colo 741 cells, which agrees with the findings of this study [23]. The results indicated that quercetin may have protective effects by downregulating eIF2α expression and promoting apoptosis in metastatic colon cancer cells.

miRNAs are single stand non-coding RNAs that suppress mRNA translation and stimulate mRNA degradation. Experimental studies have documented that irregular miRNA levels in colorectal cancer may regulate tumorigenesis [8]. Exosomes are known as vehicles for miRNAs and are miRNA biomarker

sources in bodily fluids [4]. According to our data, in comparison between control groups and quercetin application groups, total exosomal miRNA concentrations were increased in two primary and metastatic cell lines after quercetin application, but the elevations have no statistical significance.

# Conclusion

In conclusion, we have demonstrated the anticancer effects of quercetin in both Colo-320 and Colo 741 colon adenocarcinoma cells using different concentrations. Interestingly, quercetin elevated CD9 exosome marker expressions in Colo-741 cells. In addition, elevated exosomal miRNA and Dicer levels in all two Colo 320 and Colo 741 cells indicated the effectivity of quercetin. This emphasizes the possibility that decreased eIF2α expression levels could be related to the quercetin-promoted apoptosis in Colo 741 cells. However, in order to determine the exact anticancer activity of quercetin on colon cancer cells, further assessment with different molecular mechanisms and multiple biosignaling pathway molecules that include all possible cancer progression mechanisms is necessary.

# Declarations

The authors have no relevant financial interests to disclose.

### Conflict Of Interest/Competing Interests

Not Applicable.

### Data Availability

Not Applicable.

### Ethics approval

Not Applicable.

### Consent to participate

Not Applicable.

### Consent for publication

Not Applicable.

### Author's Contribution

Authors; Eda Becer, Serpil Özsoy, Hilal Kabadayı and H. Seda Vatansever prepared the research plan together. Eda Becer and H. Seda Vatansever conducted the research. Hilal Kabadayı and H. Seda Vatansever performed exosomal miRNA isolation and immuno-cytochemical analyzes. Eda Becer made

the MTT analysis of the research. Eda Becer and Serpil Özsoy made the statistical evaluation of the research. Eda Becer, Serpil Özsoy and H. Seda Vatansever compiled and wrote the research. All authors contributed to; the design of the study, the perform of the experiments, the evaluation of the results and the writing of the article.


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# Figures

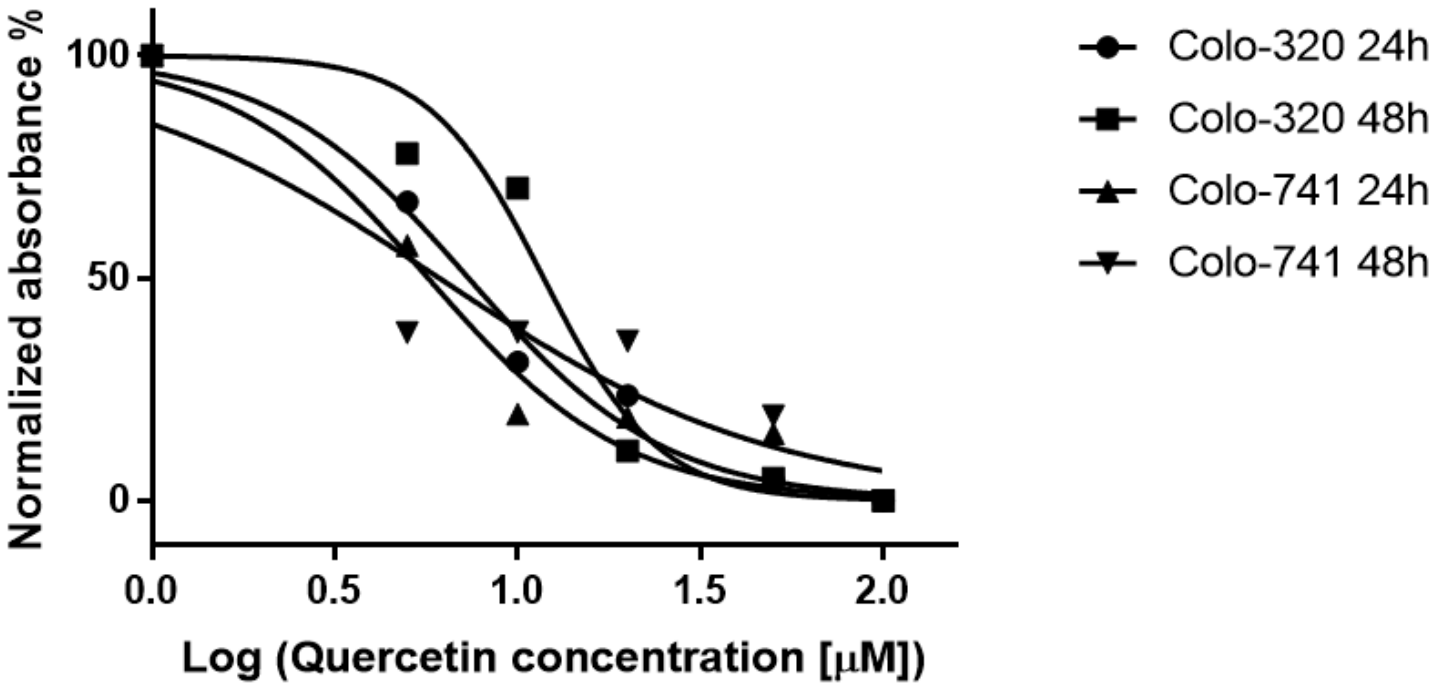



**Figure 1**

MTT assay was used for cell viability quantitation. Quercetin IC50 values were calculated for Colo 320 and Colo 741 cells. Colo 320 and Colo 741 cells were applied at different concentrations (5-100 μM) for

24 and 48h.

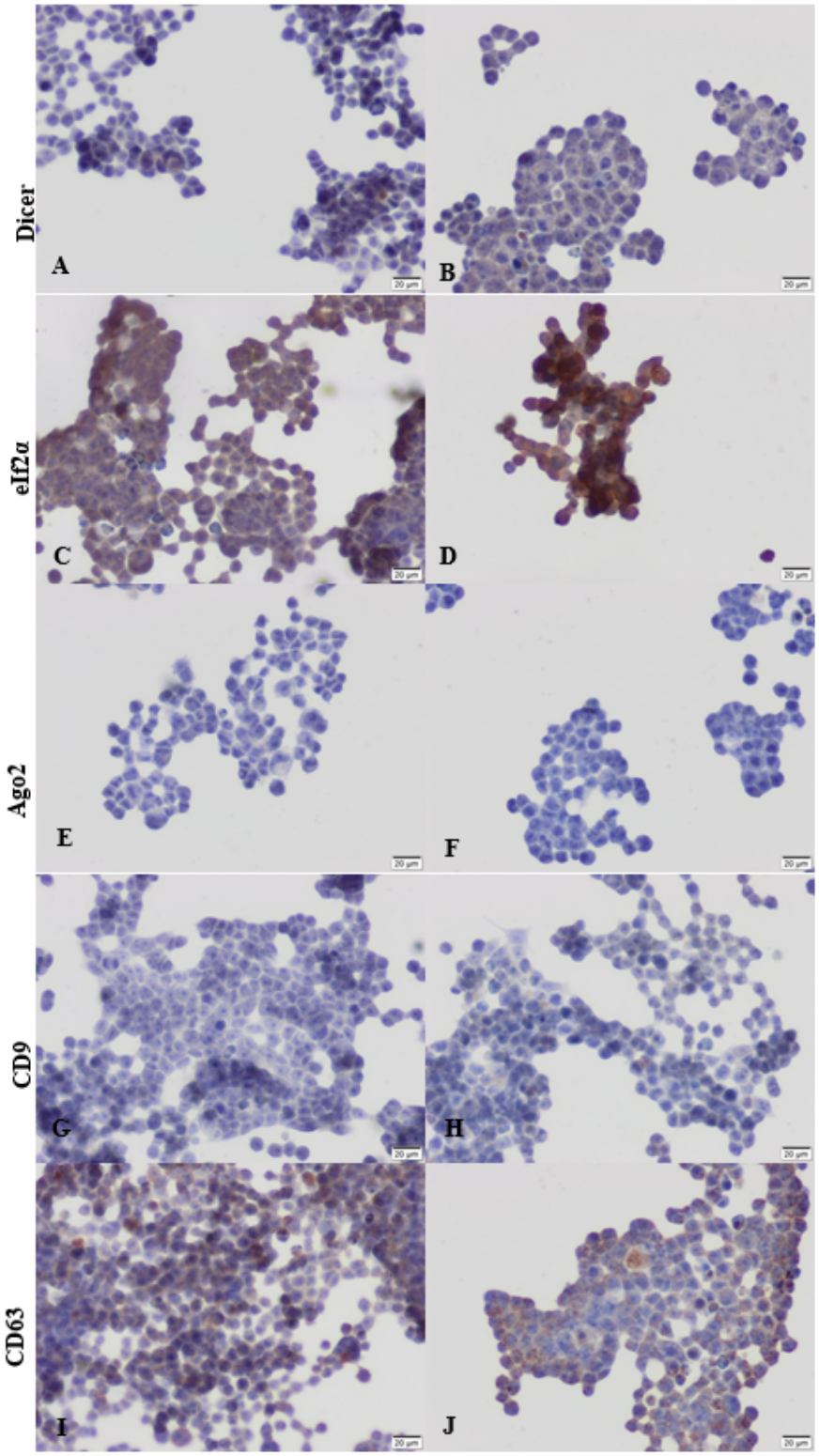
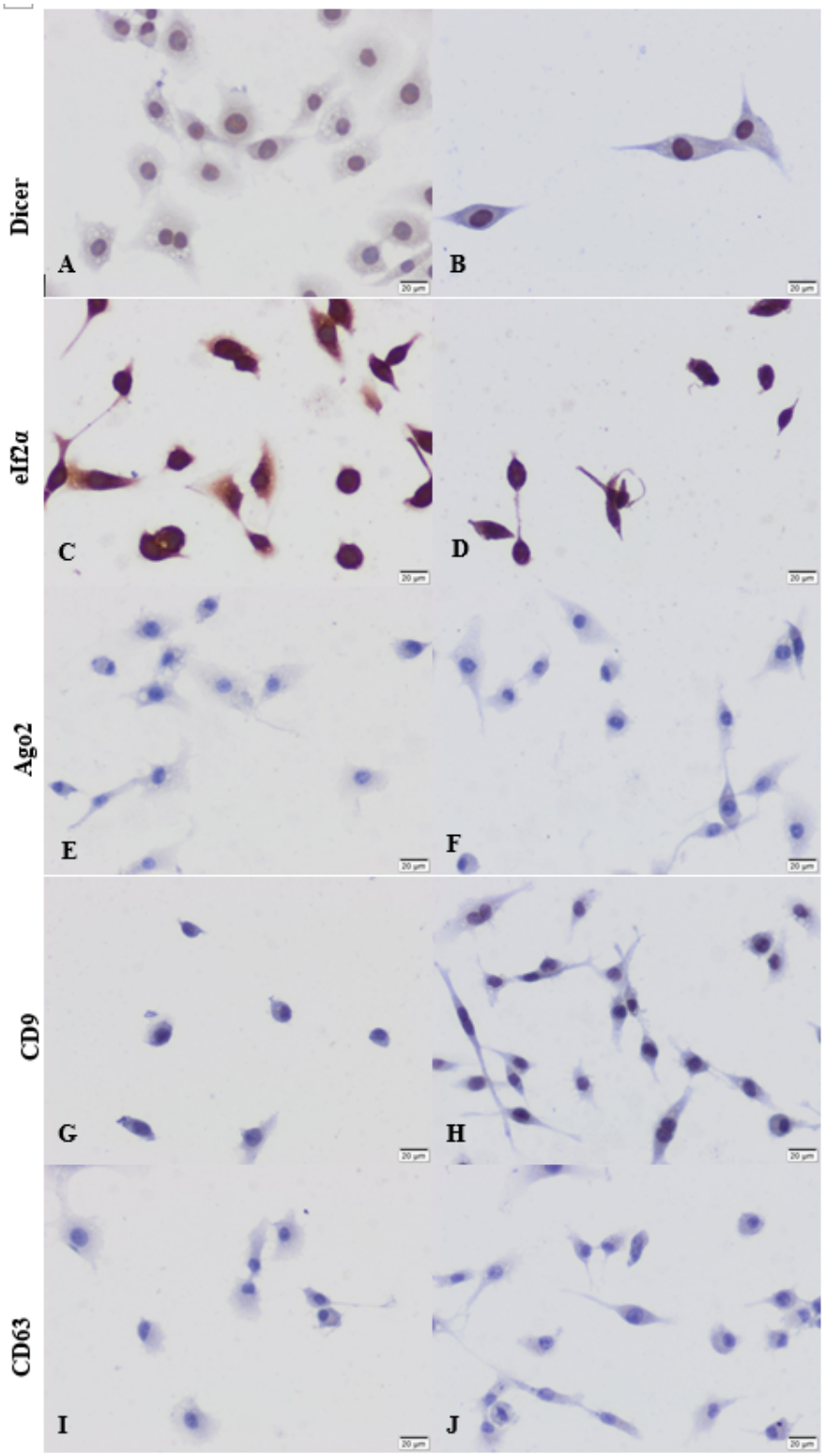



Figure 2

Immunoreactivity of Dicer (A,B), eIf2α (C, D), Ago2 (E, F), CD9 (G, H), CD63 (I, J) in control (A, C, E, G, I) and 25 μM quercetin applied (B, D, F, H, J) Colo 320 cells. Scale bars= 20μm.




## Figure 3

Immunoreactivity of Dicer (A,B), eIf2α (C, D), Ago2 (E, F), CD9 (G, H), CD63 (I, J) in control (A, C, E, G, I) and 25 μM quercetin applied (B, D, F, H, J) Colo 741 cells. Scale bars= 20μm.