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**Experimental Biomedical Research** ***Original article***

# Investigating the efficacy of different commercial forms of quercetin against breast cancer

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## ABSTRACT

**Aim**: The anti-cancer mechanisms of quercetin have been elucidated in several studies. These mechanisms are controlled by different signalling pathways within the cancer cell. This study examined the lethal effects of commercially available quercetin extracts at different doses on cancer cell lines.

**Methods:** In our study, we used 3 different commercially available supplements and looked at how well they worked against breast cancer. In our study, the presence of cell death was determined by first performing the MTT assay on the 4t1 breast cancer cell line. Additionally, cell mode was applied by staining quercetin extracts in the same commercial forms with Bax antibody to determine death mode at the same doses.

**Results:** The analysis showed that although the highest dose of quercetin (500 µM) induced apoptotic processes in cancer cells, no death was observed at the lower dose in two different supplements.

**Conclusions**: Our study results indicate that quercetin selectively inhibits cancer cell proliferation and likely induces apoptosis, making it a promising candidate for cancer therapy with potentially fewer side effects. We believe that further clinical and experimental studies are needed to elucidate its mechanisms and optimize its therapeutic application.

**Keywords**: Quercetin, supplement, flavonoids, apoptosis, breast cancer, Bax.

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## 1. Introduction

Quercetin is a natural flavonoid compound. It is found in many fruits, vegetables and plant foods (e.g. onions, apples, pomegranates, grapes and green tea) [1]. Due to its antioxidant, anti-inflammatory, anticarcinogenic and antiviral properties, quercetin has attracted a great deal of attention in health research in recent years [2]. There have also been many studies in cancer research that have investigated the potential therapeutic benefits of quercetin [3]. Quercetin has the potential to inhibit the growth, proliferation and spread of cancer cells through a number of different mechanisms.

Quercetin can neutralise free radicals in cells because it has strong antioxidant properties [1]. It is known that free radicals help cause cancer by damaging cells [4]. By preventing this type of cellular damage, Quercetin may prevent the development of cancer [5]. In addition, oxidative stress can lead to DNA damage in cancer cells. Quercetin's ability to reduce this damage may help prevent cancer cells from growing .Apoptosis is a mechanism by which healthy cells [6], spontaneously die when they are

damaged or are no longer able to carry out their functions [7]. Cancer cells are able to proliferate indefinitely because they inhibit apoptosis. In this way, it induces cancer cells to undergo apoptosis. By interacting with cellular regulatory proteins such as p53 and increasing the activation of caspase enzymes, this effect may be achieved. Quercetin prevents cancer cells from multiplying. To do so, it can target different stages in the cell cycle. Quercetin inhibits cancer cell division through cell cycle disruption [8]. Quercetin has been shown to stop some cancer cells from dividing (e.g. breast, colon and lung cancer cells) [9]. Epithelial-to-mesenchymal transition (EMT) is another factor in the development of metastasis [10]. Quercetin has been shown to reduce VEGFR-2 expression or its activation by VEGF. This can hinder the signaling cascade that would normally stimulate angiogenesis [11]. In essence, by blocking VEGFR-2 activation, quercetin can prevent endothelial cells from forming new blood vessels that tumors need to grow. After VEGFR-2 activation, a series of intracellular signaling pathways are triggered, including the PI3K/AKT/mTOR pathway [12]. This pathway is involved in cell survival, growth, and angiogenesis [13]. In prostate cancer, quercetin's ability to inhibit growth by targeting specific signaling pathways is an area of active research. One such mechanism involves the inhibition of ribosomal protein S6 kinase (often referred to as S6K), which is a key regulator of protein synthesis and cell growth. Specifically, quercetin can affect the expression and activation of S6K, influencing cellular processes that contribute to cancer cell proliferation. (P70S6K) [14]. The spread of tumours to other parts of the body is one of the deadliest features of cancer [15]. Quercetin has also been shown to have effects that prevent cancer cells from spreading (metastasizing). Quercetin may be able to inhibit the ability of cancer cells to invade (enter) tissue and prevent the growth of the tumour from spreading to other organs [5]. This study examined the lethal effects of commercially available quercetin extracts at different doses on cancer cell lines.

## 2. Materials and methods

### *2.1. Cell lines and in vitro studies*

***2.1.1. Cell line:*** The 4T1 triple negative breast cancer (TNBC) cell line was used. This particular cell line was the choice for our study because it is well known for its aggressiveness and metastatic properties.

***2.1.2. Culture media:*** RPMI-1640 supplemented with fetal bovine serum (10% FBS- Sigma F4135), GlutaMAX™ (Gibco – 35050061) and antibiotics/antimycotics (Sigma A5955) was used at 37°C and 5% $CO_2$.

100 µM- 300 µM or 500 µM quercetin extracted with methanol from different commercial products (according to [13], protocol (F1-F3) was added to each well. The weight of quercetin dehydrate (11.2 mg) was dissolved in methanol with the aid of ultrasound. A 10 mL stock solution was prepared at a concentration of 1 mg/mL. This was used to construct the calibration curve by diluting the standard solution in the range 10-500 µg/mL. Because of the stability of the quercetin solution and volatility of methanol, the solution was prepared in an aluminum foil covered amber flask. Dietary quercetin was extracted 3 times with methanol. The solvent was evaporated and dissolved in methanol. The control wells were untreated. Cells were incubated at 37°C and 5% $CO_2$ for 48 hours.

The conversion of MTT to purple formazan crystals is catalyzed by mitochondrial enzymes and is an indicator of the metabolic activity of the cell and therefore of the viability of the cell.

MTT Reagent: 10µL MTT is used at a final concentration of 0.5mg/ml, standard for many cell lines [16] 10µL of MTT labelling reagent (final concentration 0,5 mg/mL) was added to each well and plate was incubated at 37°C and 5% $CO_2$ for 3 hours. The test for cell viability was carried out according to the MTT protocol.

***2.2. Immunocytochemistry (ICC):*** Cells were treated with quercetin extracts and quercetin dihydrate at 100-300-500 µM for immunocytochemistry. The cells were incubated for a period of 48 hours. Cells were fixed with paraformaldehyde (PFA) for 15 minutes. (158127; Sigma-Aldrich)

Cells were blocked for 30 min at room temperature with a combination of sodium azide (Sigma S2002), goat serum (Sigma G9023) and Triton X-100 (Sigma T8787). Cells were probed with anti-Bax (Bax Monoclonal Antibody Thermo Fisher (6A7) (in primary antibody solution) and were incubated overnight. The next day, the cells were washed three times with 1xPBS (Sigma P4417) and treated with secondary antibody (1:1000; goat anti-mouse IgG (H+L) Alexa Fluor 488). The cells were rinsed three times with 1× PBS, counterstained with 4'6-diamino-2-phenylindole (DAPI) for 5 min, rinsed again and examined using a confocal laser scanning microscope (LSM 780, Carl Zeiss).

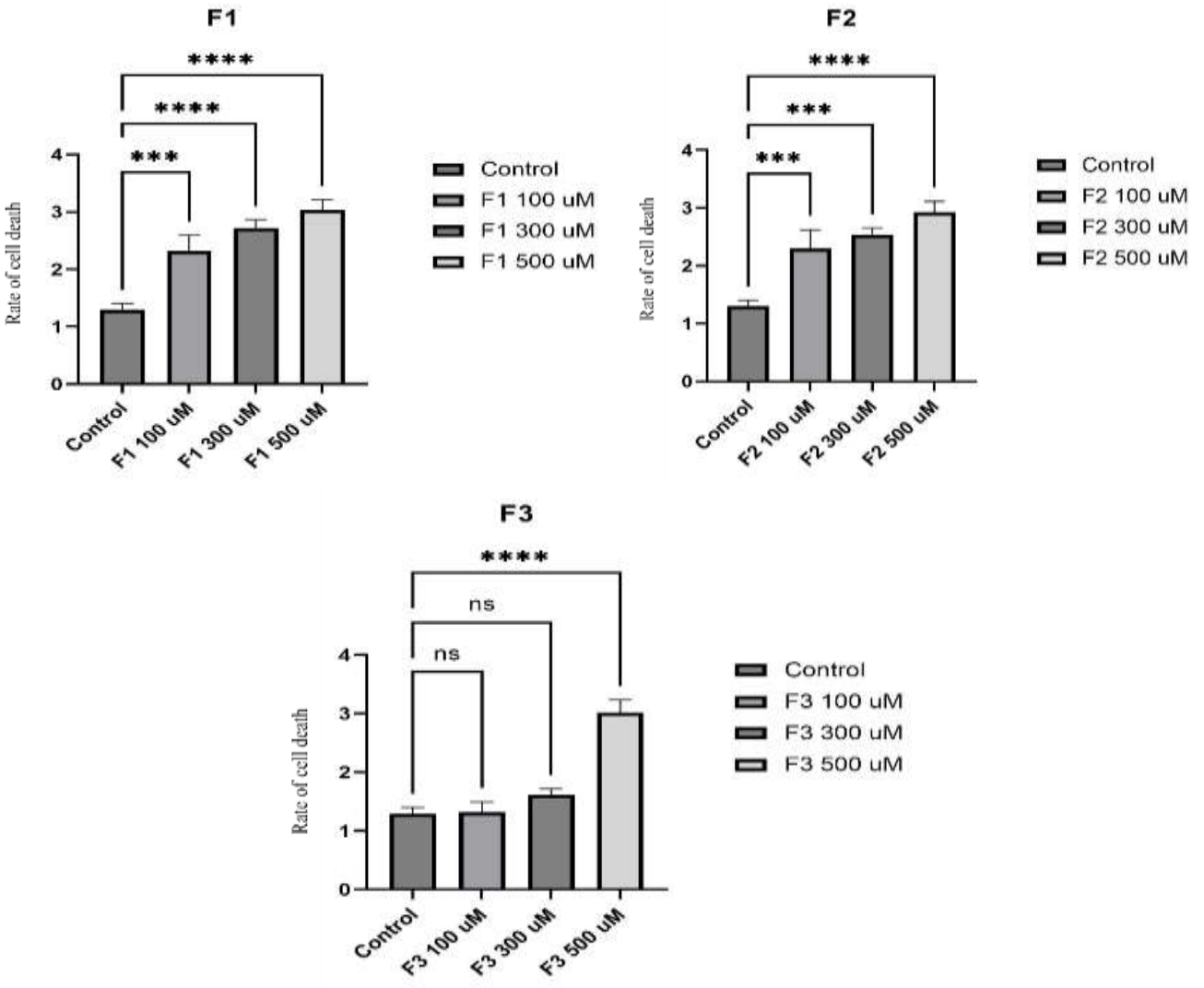


**Figure 1.** Cancer cell mortality following 48 hours incubation with commercial F1-F2 and F3 quercetin supplements *p* value <0.05.

## 3. Results

Our research focuses on evaluating the cytotoxic effects of commercially available dietary supplements containing quercetin on breast cancer cells. This approach is significant because it aims to assess the potential of quercetin as an alternative or adjunctive treatment for breast cancer, given its promising anticancer properties observed in preclinical studies.

An aggressive breast cancer cell line was treated with 100µM-300µM and 500µM quercetin in the MTT assay. In all of the commercially available quercetin products, there was a significant difference in the mortality rate at the 500 µM concentration compared to the control group. On the other hand, the mortality rate of different commercial products (F2-F3) of quercetin used at 100-300 µm concentration was significantly different compared to the control group; in the other product, it was found that the mortality rate was not significant compared to the control group**.** Differences between the two groups were ANOVA tests. Data were plotted using Prism 9.0.P value <0.05 was considered statistically significant (Figure 1).

Apoptotic cell death occurs in some tissues of the organism and are constantly formed in their cells, and this formation continues throughout life. Regulation of apoptosis is provided by the Bcl-2 / Bax gene family. Apoptotic Bax (proapoptotic) proteins after receiving the signal ion permeability of the mitochondrial membrane. They can reduce and cause the death process to start.

In our study, we observed that commercially produced quercetin at concentrations of 100-300-500 µM induced apoptotic death in cancer cells following treatment with aggressive breast cancer cells for 48 hours. In most cells, the presence of Bax protein was detected (Figure 2).

## 4. Discussion

Flavonoids have attracted attention for their health benefits (17). Flavonoids and other natural compounds have potential chemopreventive and antitumour activities because cancer incidence and mortality is still very high worldwide (18). Cancer incidence varies between regions worldwide (18).

Diet and lifestyle risk factors contribute to this discrepancy, as studies suggest. As a result, it is currently estimated that many appropriate lifestyle changes, especially changes in diet or nutrition, may prevent cancer deaths (19). With anti-inflammatory and chemopreventive effects, quercetin is the most important bioflavonoid in the human diet (20). It also modulates the regulation of proliferative and antiapoptotic proteins. There is evidence from many studies that quercetin is an inducer of cancer cell death (21).

One of the deadly features of cancer is that the tumour spreads to other parts of the body (22). Quercetin has also been shown to have effects that prevent the spread (metastasis) of cancer cells to other parts of the body. It can reduce the ability of cancer cells to invade and spread to other organs (14). It is still unclear what the anti-tumour mechanism of quercetin. There are only a few studies on quercetin extracts and quercetin dihydrate forms. Therefore, we investigated their effects in both 4T1 cells and 4T2 cells. We suggest that quercetin has potential as an alternative or adjunctive treatment for breast cancer, given the promising anticancer properties observed in preclinical studies.

Akt and STAT3 are two critical signaling pathways (12). They support and cooperate with each other in cancer cells. Both are involved in regulating cell growth, survival and metastasis. Akt can activate STAT3, which can increase the expression of genes that help cancer cells grow

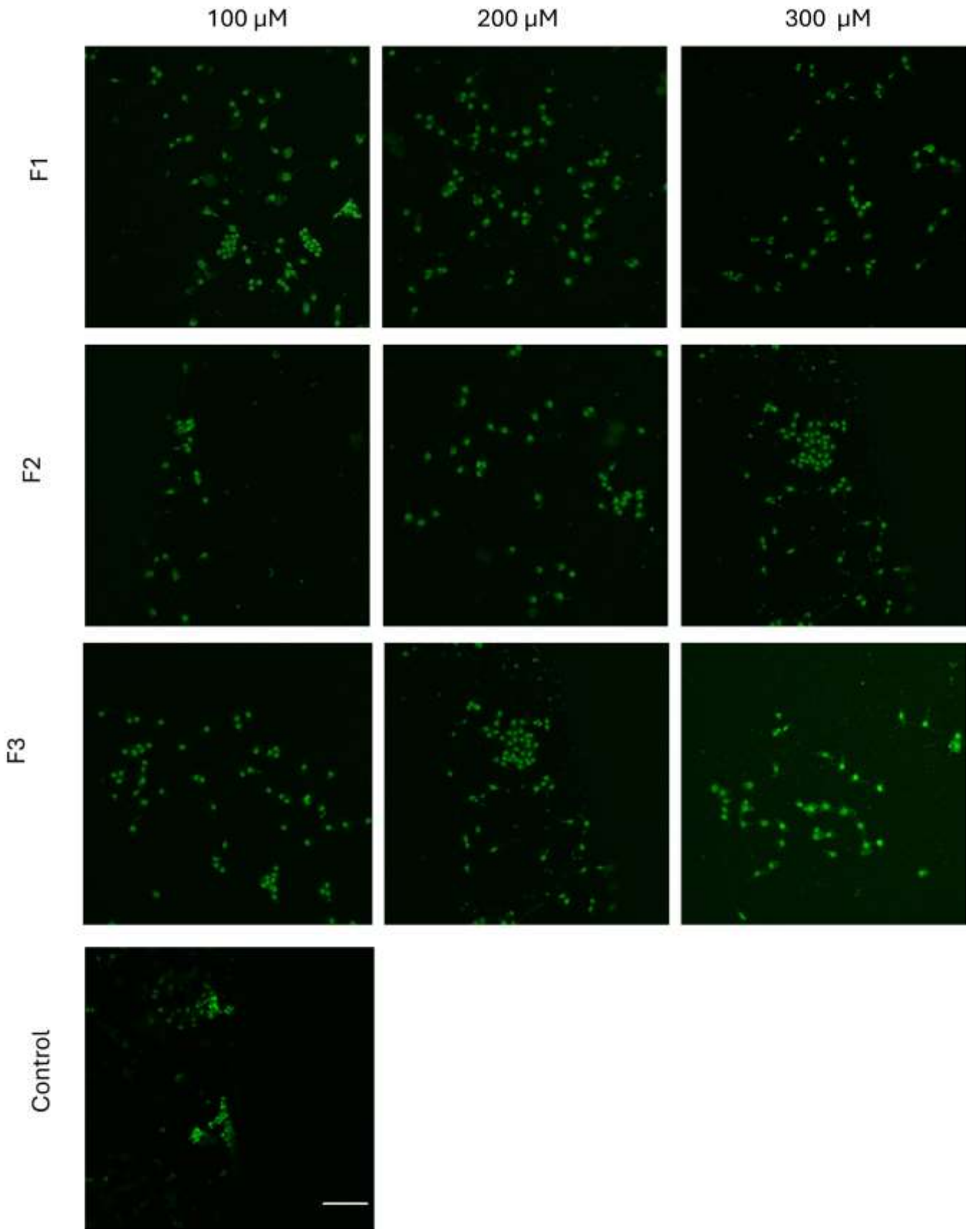


**Figure 2.** Quercetin was obtained in 3 different concentrations from 3 different commercially available dietary supplements. It was observed that quercetin induced tumour death by apoptosis of the Bax protein. (Scale bar 50 µm).

and survive (8). This means that Akt and STAT3 work together to control how cancer develops. Quercetin's ability to inhibit Akt and STAT3 suggests that it has a versatile mechanism for treating cancer (21). By blocking activation of Akt and STAT3, it prevents cancer cells from multiplying. In our study, we believe that quercetin triggers cell death by activating this mechanism in the experimental groups where there is a high level of cell death.

In cancer therapy, the potential of quercetin to modulate the IL-6/JAK2/STAT3 pathway is being investigated (23). By reducing IL-6 levels, quercetin may prevent activation of the IL-6/JAK2/STAT3 pathway. This may slow cancer cell growth and prevent metastasis (24). Quercetin's anti-tumour immune effects on triple negative breast cancer were investigated using 4T1 cells and a 4T1 cell xenograft mouse model (23). Quercetin was shown to inhibit 4T1 cell proliferation, migration and invasion and suppress the IL-6/JAK2/STAT3 signalling pathway (23).Quercetin extracts and dihydrate forms are used in this study but are not in commercial ones. In our study, we added the commercial forms of quercetin and examined the effects they had on cancer cells.

Despite quercetin's promising anti-cancer properties, its short biological half-life significantly limits its use in cancer treatment (1). Different commercial products do not show the same effect at the same doses as in our study. While low doses of quercetin have effects such as disease, cell isolation and isolation, high doses are expected to induce apoptosis and cell destruction. This study will be able to show the extent to which different doses trigger this structure. This may be crucial for research into the bioavailability and efficacy of quercetin. Different commercial forms of quercetin may have different effects on how quercetin is absorbed and acts in the body. Absorption rates and biological activity may differ between commercial forms. This may affect the treatment potential. As a matter of fact, this is what our study proves. This information will be used to investigate the cancer cell death pathways activated by quercetin and other proteins and transcription factors. It is also planned to create a tumour model in laboratory animals to study the efficacy of quercetin under in vivo conditions.

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***Ethical Statement:*** *Since this was a laboratory study, an ethics committee decision was not required.*



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