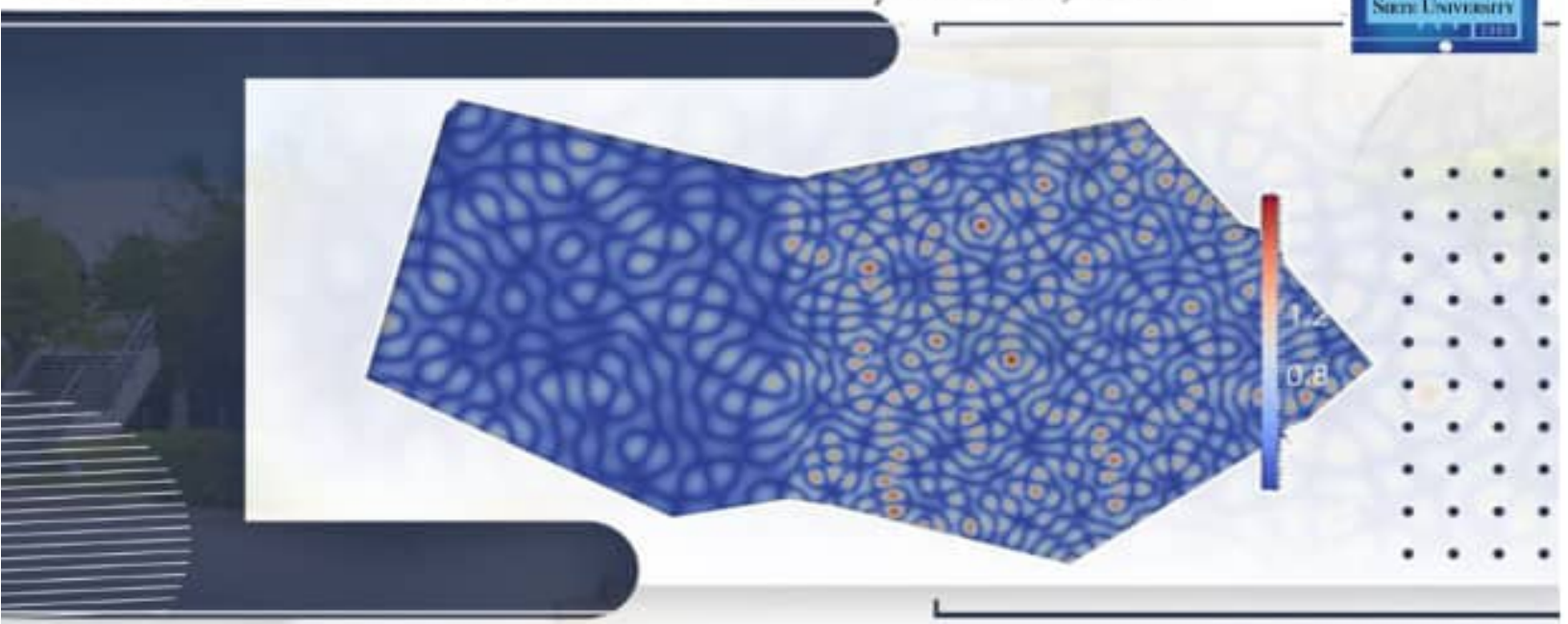
eISSN: 2789-858X

Scientific Journal for the faculty of Science - Sirte University

DOI: 10.37375/issn.2789-858X - Indexed by Crossref, USA



Volume2 Issue2 October 2022

Bi-annual Peer-Reviewed, Indexed,and Open Accessed e-Journal

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Scientific Journal for the Faculty of Science-Sirte University Vol. 2, No. 2 (2022) 19-27

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

Anticancer Screening of Quercetin as a Natural Treatment

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DOI: https://doi.org/10.37375/sjfssu.v2i2.439

ARTICLE INFO:

Received 14 June 2022.

Accepted 21 September 2022.

Published 27 October 2022.

Keywords:

Oxidation stress, Anticancer, Quercetin, Apoptosis, Antioxidant

A B S T R A C T

Oxidation stress is a process that damages the cells of the body, and also leads to the happen of many diseases such as cancer. This disease is known among all ages in every part of the world. In cancer cells, increasing types of free radicals flaw the balance in the cell and thus increasing free radicals target all types of molecules, including proteins, lipids, and nucleic acids. If the body cannot produce enough antioxidants, increasing free radicals damage the cells of the body. The defect in apoptosis also prevents malignant cells from being damaged. This paper aims to study the effect and activity of quercetin as an antioxidant and anticancer drug in vitro. The mechanism of increasing free radical formation causing cell damage will be explained. This study also presents a discussion about the mechanism of apoptosis pathways using the MTT scale to measure the cell's ability to metabolic activity and whether the cells are still alive. The most significant shifts were for (Raji, MOLT-4 and CT-26 equal to 0.18 ± 0.09, 2.1 ± 0.9, and 5.5 ± 0.38, respectively. Apoptosis was detected using the BD Annexin V FITC assay and apoptosis was measured at ($P < 0.001$). Also, in vivo, the positive effects of different doses of quercetin on affected models are discussed.

1 Introduction

Under normal circumstances, a person's life continues with health problems. Wrong practices such as smoking and environmental pollutants lead to the production of harmful things in the body called free radicals (Phaniendra et al., 2015). When these unstable free radicals attack the body, the body produces antioxidants to protect it. If the body cannot produce enough antioxidants (Shi et al., 2019), free radicals damage the cells of the body.

Atoms are stable by filling orbitals with electrons. When the orbital is filled with electrons, the additional number of electrons shift to the next path. If the electrons ends, and at the same time the outer orbit of the atom is not filled with the required number of electrons, then this atom is attached to another atom to complete the outer orbit of the atom's electrons. In this case, the atom with an incomplete outer shell is known as free radical (Phaniendra et al., 2015).

Free radicals are unstable atoms react quickly with other materials (Phaniendra et al., 2015), (Widowati et al., 2013) For example, when oxygen molecules split into single atoms that do not have double electrons, they become unstable free radicals intended to bind to other molecules (Tavsana and Kayalia, 2019), (Prieto-Bermejo and Hernández-Hernández, 2017). If these steps continue to occur, the process can be called oxidation stress (Pham-Huy et al., 2008). The process of oxidation stress damages the cells of the body, and also leads to many diseases, such as cancer. Free radicals target all types of molecules, including proteins, fats, and nucleic acids. When a person gets older, the body loses its ability to fight the effects of free radicals (Phaniendra et al., 2015), (Pham-Huy et al., 2008). This leads the body to more free radicals and more oxidation stress (Widowati et al., 2013), (Pham-Huy et al., 2008). The danger of " oxidation stress" is that free oxygen atoms reach the genetic material in DNA cells, affecting their structure

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and causing a mutation in the cell, which turns into diseased cells or malignant cancer cells (Phaniendra et al., 2015), (Prieto-Bermejo and Hernández-Hernández, 2017).

Free radicals which call reactive spices are produced in the human body and are an internal source for the metabolism process (Tavsana and Kayalia, 2019), but there are many lifestyle factors that can accelerate the production of these free radicals, such as exposure to toxic chemicals, drugs, and pollutants. These factors are called external sources. These free radicals caused by accelerating agents are called hyperreactive spices (Prieto-Bermejo and Hernández-Hernández, 2017). The oxidation (pro- oxidation) reaction occurs when free radicals interact with any molecule that collides with them (Pham-Huy et al., 2008). This process disables free radicals, but turns the other molecule into new free radicals. This is how a free radical chain reaction starts, damaging all of the molecules it interacts with. When the molecules are damaged, cells stop working and die (Pham-Huy et al., 2008).

In cancer cells, oxidation stress occurs with the increase in oxidation processes (Phaniendra et al., 2015), (Pham-Huy et al., 2008). Normal, uninfected cells contain reactive types of oxygen that modulate cell function. Whereas in cancer cells, these types are increasing, and they facilitate imbalances in the cell (Pham-Huy et al., 2008).

The free radical oxidation process can be controlled by antioxidants (Shi et al., 2019), (Pham-Huy et al., 2008). Antioxidants are chemicals that work against free radicals. It also prevents free radical oxidation, and donates electrons to free radicals to become stable (Shi et al., 2019), (Pham-Huy et al., 2008). The benefit that makes antioxidants unique is to donate an electron without becoming free radicals. Different types of food act as antioxidants, and the body also produces some antioxidants, such as vitamins (Shi et al., 2019), (Pham-Huy et al., 2008).

The main antioxidants found inside the cells of the body are enzymes, the non-enzymatic component called glutathione and various types of oxidase (Shi et al., 2019). These antioxidants eliminate reactive oxygen species (ROS). For example, (ROS) are eliminated by "superoxide dismutases (SOD) (Tavsana and Kayalia, 2019), (Prieto-Bermejo and Hernández-Hernández, 2017), glutathione peroxidase (GPx), (Phaniendra et al., 2015) peroxiredoxins (PRDXs), glutaredoxins, thioredoxins (TRXs), and catalases." Also, these enzymes and catalases are found within mitochondria. For example, enzymes convert the free-radical O_{2}^{-} anion into hydrogen peroxide and then discard it (Phaniendra et al., 2015), (Prieto-Bermejo and Hernández-Hernández, 2017), (Pham-Huy et al., 2008).

Cells contain a number of components which are mitochondria, endoplasmic reticulum and peroxisomes. Each of these three components does its job. Within all these organs there are different types of enzymes that have oxidation activation (Pham-Huy et al., 2008). These enzymes react with oxygen molecules to produce radicals. In the body of an organism, during the process of metabolism, a number of reactive free radicals are produced using various reactions (Tavsana and Kayalia, 2019), such as the endogenous oxidation reaction and non-enzymatic reactions. These free radicals have one non-double electron, such as O_{2}^{*-}, $RO^{\cdot}$, $ROO^{\cdot}$, $OH^{\cdot}$ and ... etc. All of them are highly efficient, and their half-life is 10^{-6} s, 10^{-6} s, 10^{-7} s, 10^{-10} s respectively (Phaniendra et al., 2015). For example, in mitochondria, the radical of anions O_{2}^{*-} and H_2O_2 can be formed by oxides enzymes under physiological conditions (Phaniendra et al., 2015), (Prieto-Bermejo and Hernández-Hernández, 2017).

$$O_2 + Fe^{+2} \rightarrow Fe^{+3} + O_{2}^{*-} \quad .....(1)$$

$$O_2 + O_{2}^{*-} + 2H_2O. \rightarrow H_2O_2 + O_2 \quad .....(2)$$

Cells are more susceptible to damage due to the formation of new hydroxyl radicals resulting from the reaction of anions O_{2}^{*-} and H_2O_2 as equivalent. (3) (Phaniendra et al., 2015).

$$O_{2}^{*-} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^{-} .....(3)$$

While H_2O_2 damaged by "catalase, glutathione peroxidase and peroxiredoxins enzymes", glyceraldehyde-3-phosphate enzyme cannot function as intended, so it stops when H_2O_2 is in high concentrations (Phaniendra et al., 2015). This is because it produces $OH^{\cdot}$ which damages DNA action. 8-Hydroxydeoxyguanosine is the product of many chemical reactions that take place between the hydroxyl radical and the components of DNA (Phaniendra et al., 2015). Due to the number of changes that occur in the immune system, 8-hydroxydeoxyguanosine is responsible for a number of abnormalities that cause cancer. High levels of 8-hydroxydeoxyguanosine is produced within mitochondrial DNA (Prieto-Bermejo and Hernández-Hernández, 2017), because increasing types of free radicals are produced inside mitochondria whereas in nucleic DNA, the levels are low (Phaniendra et al., 2015), (Shi et al., 2019), (Pham-Huy et al., 2008).

RNA is produced in the nucleus and secreted into the cytoplasm to stay there. As the RNA is demolished and then rebuilt, the DNA will be stable (Prieto-Bermejo and Hernández-Hernández, 2017). There is a great deal of

damage to RNA in the cytoplasm due to oxidation stress that results in increasing free radicals. Damage from oxidation stress to RNA is more than that from DNA. (Phaniendra et al., 2015), (Prieto-Bermejo and Hernández-Hernández, 2017). This damage is due to the fact that the RNA is close to the mitochondria, which is the right place for generating excess types of oxygen (Phaniendra et al., 2015).

The human body is prepared to deal with cancer cells (Nicholson, 2016). To create new cells, these cells are in a continuous state of reincarnation (Nicholson, 2016). Every second, more than ten million new cells are created by transcription, replacing others that have permanently terminated their activity. Hence, the cells have a mutation during transcription. Besides, cells are constantly exposed to substances that destroy and damage DNA. DNA is the main component of the cell's genetic map and is responsible for transcription (Prieto-Bermejo and Hernández-Hernández, 2017). This problem and damage is caused by unstable free radicals during the metabolism process. Cancer develops when there is an imbalance between antioxidants and oxidants. Major damage to the immune system, proteins and fats is caused by the accumulation of free radicals that are caused by metabolism (Pham-Huy et al., 2008).

The immune system damages the abnormal cells right before the cells can multiply (Pham-Huy et al., 2008), (Nicholson, 2016). It contains white cells that damage these abnormal cells before they take any chance to reproduce. Responsibility does not depend on the immune system alone, but there is a special program within each cell that identifies any abnormality or mutation that occurs during reproduction (Pham-Huy et al., 2008), (Nicholson, 2016). Most of the time, the protective mechanisms within the body are able to prevent the happen of cancer (Pham-Huy et al., 2008). However, the immune system sometimes loses its ability to recognize and damage cancer cells, and these cells begin to multiply uncontrollably. On the other hand, when there is a large number of abnormal cells, the immune system cannot do its job (Nicholson, 2016).

1.1 Mechanism of reaction to form (ROS): (Phaniendra et al., 2015) (Tavsana and Kayalia, 2019), (Prieto-Bermejo and Hernández-Hernández, 2017).

There are a number of pathways used to transfer electrons within mitochondria. Complex I and Complex II or III are the main paths (Phaniendra et al., 2015). As a result of this transfer of electrons shown in Figure (1), the rate of free radical production increases with the increase in the rate of metabolic processes, as some of these radicals turn into hydrogen peroxide which has high toxicity due to the activation of mitochondrial superoxide dismutase (Phaniendra et al., 2015). In the absence of catalase or glutathione, hydrogen peroxide remains in the cells and causes damage to the tissues of the organism (Phaniendra et al., 2015).

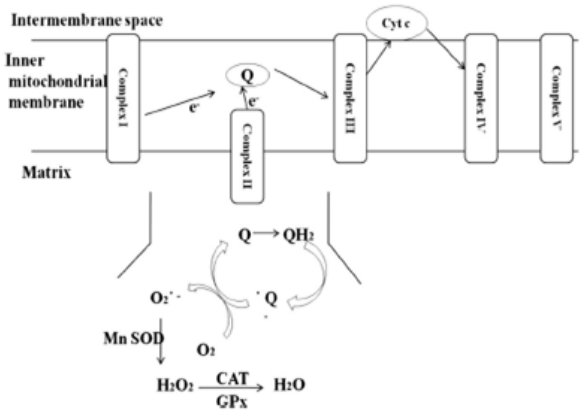

Figure (1): Production of (ROS) and their toxicity inside of mitochondria.

One of the main and natural things that happen in the tissues of the human body is that the cells of the body are in a state of constant renewal (Abcam, 2016). By dividing cells, new cells are formed. For example, skin cells are damaged over time and replaced with new cells. Also, the fragmented small particles are eliminated by neighboring cells. This ordered process is called apoptosis (Phaniendra et al., 2015), (Abcam, 2016), (Xiong, 2014), (https://www.cancerquest.org/cancer-biology/apoptosis, 2020). There are three important pathways that occur to initiate the apoptotic process of cell death, the death receptor pathway, the mitochondrial pathway, and the "Perforin / Granzyme" pathway. In the first pathway, death signals are sent inside the cell by a series of proteins. These proteins perform a number of tasks, the most important of which is caspase activation (Phaniendra et al., 2015), (Xiong, 2014), (https://www.cancerquest.org/cancer-biology/apoptosis, 2020).

In the second pathway: the proteins of the Bcl-2 family have various functions such as stimulating apoptosis and at the same time acting as an anti-apoptotic, and the proteins of the Bcl-2 family can tell which signal before apoptosis occurs (Xiong, 2014). For example, BH3 proteins are part of the most important proteins in the Bcl-2 family, and have a unique function that activates the "Bax or Bak" proteins. The "Bax or Bak" proteins are called pro-apoptotic proteins (https://www.cancerquest.org/cancer-biology/apoptosis, 2020). Also, BH3 proteins prevent "Bax or Bak" proteins from being anti-apoptotic proteins. As a result of the permeability of the outer mitochondrial membrane (Xiong, 2014), an apoptotic factor called Cytochrome c is excreted from the mitochondrial membrane into the cytosol. A number of compounds are produced, such as

"apoptosome" and "Apaf-1", which activate a series of caspases. These caspases are enzymes that damage required cells as shown below (Xiong, 2014).

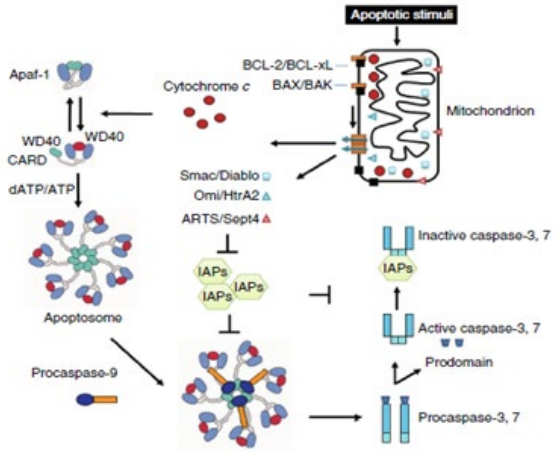

Figure (2). Activation of caspases by mitochondria pathway (Xiong, 2014).

In the last pathway: the purpurin protein is produced by lymphocytes. This protein can open small pores in the plasma membrane (Xiong, 2014). Lymphocytes also work to produce small molecules that can enable these particles to enter these pores and secrete a number of enzymes, such as Granzyme that complete the implementation process and activate chains of caspases (https://www.cancerquest.org/cancer-biology/apoptosis, 2020). The implementation stage is one of the last stages that cannot be stopped upon implementation. When the orders are damaged, the cells are damaged by a number of Caspases. Caspases damage desired cells. The surrounding cells are swallowed, digested, and permanently disposed of (Xiong, 2014).

Cells can decline and die in multiple ways. Most cells in vertebrates die by the mitochondrial pathway. The mitochondrial pathway begins with a wide range of signals such as DNA damage, growth factor deprivation, and more (https://www.cancerquest.org/cancer-biology/apoptosis, 2020). The changes that transform a normal cell into a malignant cell. It is simple. When cancer cells proliferate, cell death (apoptosis) decreases (https://www.cancerquest.org/cancer-biology/apoptosis, 2020). In fact, cancer cells inhibit the apoptotic response, as it is the defect in apoptosis that prevents malignant cells from being killed. Induction of apoptosis by inhibition of cell proliferation was determined by Widowati et al.. The defect in apoptosis can be treated with quercetin using the caspase-3 activation mechanism and regulation of a number of enzyme pathways (Xiong, 2014).

1.2 Quercetin

A healthy diet rich in antioxidants plays an important role in fighting free radical damage (Ramalalingam et al., 2015), (Niu et al., 2011), (Mukherjee et al., 2019). Polyphonics have been isolated from many types of natural foods such as vegetables, fruits, nuts, peels, roots, etc. These compounds are called flavonoids. They have biological activities (Ramalalingam et al., 2015), (Niu et al., 2011). For example, quercetin as shown below in Figure 3 is one of the most abundant antioxidants in the human diet and can be a drug that prevents and treats different types of cancers (Niu et al., 2011), (Mukherjee et al., 2019). It is part of the flavonoids that can use a unique mechanism. Quercetin is metabolized within the gastrointestinal tract, and can be absorbed into the membrane of the small intestine without using the hydrolysis process (Mukherjee et al., 2019). Therefore, the focus of a number of previous studies was on extracting many natural substances that act as antioxidants and anti-cancers. For example, the results of the current studies conducted by Ramalalingam et al. were for the extraction of chloroform from the leaves of Aristolochia indica L, and quercetin was also extracted for its antioxidant and anti-cancer properties by Guomin et al. and Mukherjee et al.

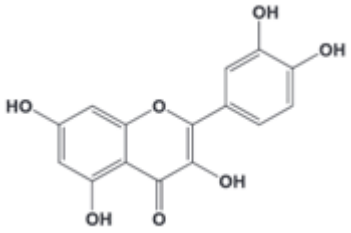

Figure (3). Chemical structure of Quercetin (Mukherjee et al., 2019).

Quercetin has a total antioxidant capacity, TAC, which inhibits the activity of excessive oxygen species caused by the presence of diethylnitrosamine, which is one of the components of cigarette smoke that causes hepatocellular carcinoma. Apoptosis has several pathways that quercetin can activate such as the pathway in MDA-MB-231 cells through the diffusion of the calcium signal to mitochondria. Also the activation of many caspases, the activation of the intrinsic pathway in HepG2 cells, thus "quercetin promotes translocation of Bax from cytosol into mitochondria membrane leading to depolarization of mitochondrial membrane" and the resulting response to the control of some signals as shown in Figure 4. The rest of the other mechanics are available in the study of Rather and Bhagat, 2020.

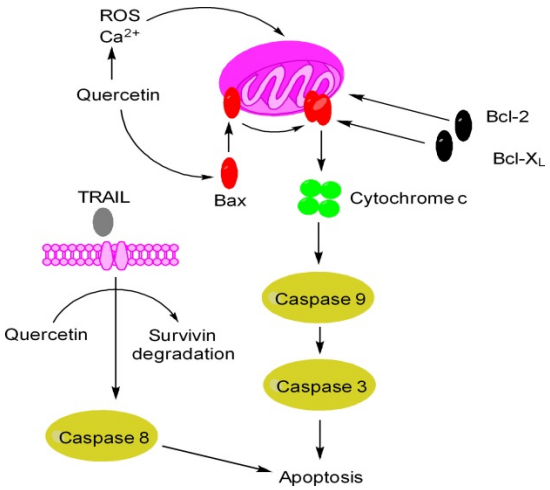

Figure (4): Mechanism of Quercetin as therapeutic medicine (Rather and Bhagat, 2020)

2. Methods

2.1 MTT Assay:

It is a test to measure cell viability and metabolic activity. In the cytosol inside the cell, this assay depends on the use of dimethylsulfoxide (DMSO) as a polar aprotic solvent, and also depends on the nicotinamide adenine dinucleotide phosphate (NADPH) that is "a required cofactor, used to donate electrons and a hydrogens to reactions catalyzed by some enzymes". (Ali-Boucetta et al., 2021), (Hingorani et al., 2011), (Hashemzael et al., 2017). Cells must be alive to convert MTT, which is a yellow (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) to insoluble purple formazan. DMSO solvent dissolves purple formazan (not dissolved in water) and convert them into a colored solution. Also, the MTT scale is used to check cell sensitivity by toxic drug effects on cell lines (Hingorani et al., 2011). Because of the light sensitivity, this test is performed in the dark. The absorbance of the colored solution is determined using a spectrophotometer (between 500 and 600 nm), (Hingorani et al., 2011). The degree of light absorption depends on the accumulation of formazan inside the cell and on the surface. When the concentration of Formazan increases, the violet color increases and the absorbance becomes less. This indicates the survival of the cell (Hingorani et al., 2011), (Jiao et al., 2015).

2.2 Measure cell viability Method (Ali-Boucetta et al., 2021), (Hashemzael et al., 2017), (Jiao et al., 2015):

1. Use a dilution chain (10, 20, 40, 80 and 120 µM)) of quercetin to test (MTT) and prepare the MTT solution.

2. All 9 cancer cell lines (shown in table 1) are classified and treated with a set of concentrations in triplicate in 96 plates and incubated overnight.

3. Dilute the MTT and add the chain (10, 20, 40, 80 and 120 µM) of quercetin

4. Cells were coated in 96 well plates at 37˚C for 72 hours.

5. After incubation, the wells were suctioned and the dye dissolved in DMSO

6. Absorption was measured at 570 nm.

7. Compared to untreated control cells.

8. The percentage of cell survival is calculated using this formula:

$$\% \text{ Cell viability} = \frac{A_{570nm} \text{ of treated cells}}{A_{570nm} \text{ of untreated cells}} \times 100.$$

2.3 The BD Annexin V FITC Assay: (Hingorani et al., 2011),[19]

In the healthy cell, membrane phosphatidylserine (PS) is located on the inner plasma membrane, but when the phosphatidylserine (PS) is transported from the inner side of the plasma membrane to the surface, the plasma membrane is completely lost, and it becomes an invalid cell. Because of the electronic affliction, "Annexin V, a Ca2+-dependent phospholipid-binding protein" can bind to the cellular phosphatide membrane on the outer surface of the cell's plasma membrane. The BD Annexin V FITC Assay on the BD FACSVerse™ System used to detect the location of membrane phosphatidylserine (PS), which caused apoptosis.

2.4 Measuring apoptosis by tracking cells (Abcam, 2016), (Hingorani et al., 2011), (Jin et al., 2019).

There are two processes that work to determine the difference between cells that have had early death and those that have a late death are shown in figure 5-a .

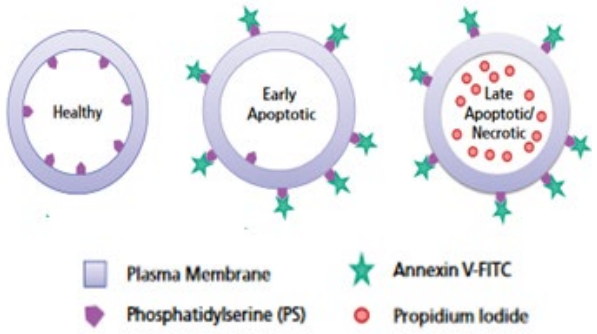

Figure (5-a): Diagram shows The healthy and apoptotic cells. (Abcam, 2016), (Hingorani et al., 2011).

The process of staining with Annexin V is called early apoptosis and the process of using propidium (PI) is termed late apoptosis. Consider the following:

1- Viable cells with healthy membranes cannot be stained with the use of Annexin V or penetrate the propidium dye (PI) so that the expression used for the description will be negative-negative (Foo et al., 2019), while in late apoptosis the membranes of dead cells penetrate the PI and the expression Annexin V and PI is positive – positive (Hingorani et al., 2011).

2- In early apoptosis with intact membranes will be Annexin V and PI positive - negative While the death stage apoptosis the expression used for description is Annexin V and PI positive as shown below in Figure 5-b (Hingorani et al., 2011).

2.5 Detection of Cell Apoptosis Method (Hingorani et al., 2011):

1- The affected cells were implanted into 12-well plates (2 x 105 cells / well).

2- The cells were incubated (at 5% CO2 and 95% air) at 37 ° C.

3- DMSO was used to dissolve quercetin in the following concentrations (10, 20, 40, 80 and 120 µM)

4- After the cells were incubated for 48 hours in the dark, the cells were analyzed.

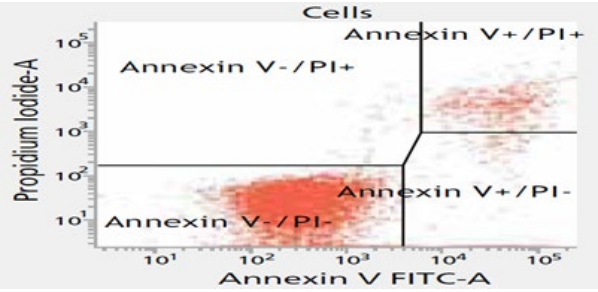

Fig (5-b). Using the BD Annexin V FITC assay on the BD FACSVerse™ System to detect apoptosis (Hingorani et al., 2011), (Foo et al., 2019).

3. Results

The MTT assay was performed to assess the viability of all nine tumor cell lines illustrated in table 1 applied for 24, 48 and 72 h. Using three doses of quercetin (10, 40 and 120 µM) for the treatment purpose illustrated in Table 2 (Hashemzael et al., 2017). At the time of 24 h, there was no inhibition at a concentration of 120 µL for both PC3 and CHO cells, respectively, and at the same time, the inhibition of all other cells was apparent in Table 2. This means that the most significant shifts will be for (Raji, MOLT-4 and CT-26 equal to 0.18 ± 0.09, 2.1 ± 0.9, and 5.5 ± 0.38, respectively, while the least significant shifts are observed for PC3 and LNCaP equal to 31.5 ± 3.7 and 30.7 ± 2, respectively.

Table (1). Symbols used for cancer cell lines

Name of cancer cell lines	Symbols of lines
Colon carcinoma cells	CT-26
Prostate adenocarcinoma cells	LNCaP
Human prostate cells	PC3
Pheochromocytoma	PC12
Michigan Cancer Foundation-7, estrogen receptor positive breast cancer cells	MCF-7
Acute lymphoblastic leukemia cells	MOLT-4
Human myeloma cells	U266B1
Human lymphoid cells	Raji
Ovarian cancer cells	CHO

The use of 120 µM of quercetin had an effect on the rate of apoptosis of CT-26, LNCaP, MOLT-4 and Raji cell lines, and therefore the apoptosis of these lines was significant compared to the control group ($P < 0.001$).

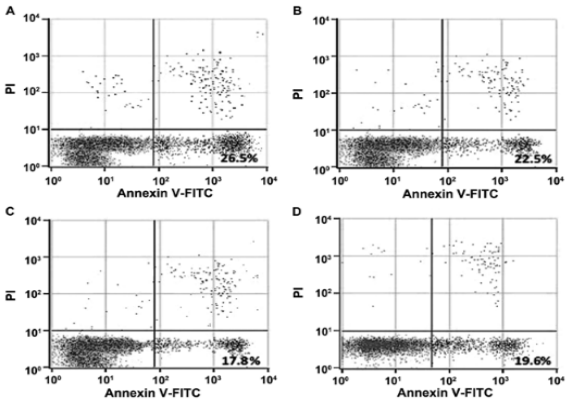

Figure (6). Apoptotic rate determined by Annexin V/PI staining in (A) CT-26, (B) LNCaP, (C) MOLT-4 and (D) Raji cell lines following 48 h of treatment with quercetin at 120 µM. Early apoptotic cells are Annexin V-positive and PI-negative (lower right quadrant). PI, propidium iodide (Hashemzael et al., 2017).

Figure 6 and 7 examine the rate of apoptosis in 4 cell lines "CT-26, LNCaP, MOLT-4 and Raji, respectively" that have high and low sensitivity to quercetin. In these cells, quercetin initiated the apoptosis process in a dose-dependent manner. The results of the MTT assay were in agreement with the Annexin V/PI assay, as the cell lines showed extreme sensitivity to quercetin at high concentrations that led to apoptosis at ($P < 0.001$) (Hashemzael et al., 2017).

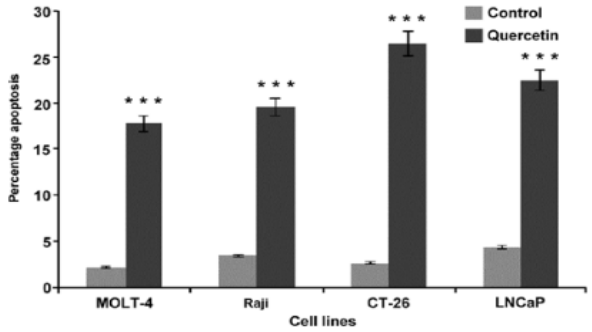

Figure (7). Induction of apoptosis in LNCaP, CT-26, MOLT-4 and Raji cell lines treated with quercetin, (Hashemzael et al., 2017).

While the effect of quercetin doses (10, 20, 40, 80 and 120 µM) was great on cell lines in vitro, these doses of quercetin did not affect the in vivo. Therefore, different concentrations of quercetin (50, 100 and 200 mg/kg) were used on Mice bearing CT-26 and MCF-7 tumors. The treatment was completed and the tumor size decreased on days 18 and 20 as shown in Figure 8.

Table (2). Comparison of Hashemzael et al. data obtained from the MTT assay by using doses of quercetin applied to different cancer cell lines for 24, 48 and 72 hour**.**

Cell line	24h			48h			72h		
	10 µM	40 µM	120 µM	10 µM	40 µM	120 µM	10 µM	40 µM	120 µM
CT-26	94.2±4.4	75.1±4.2	49.7±5.9	87.4±5.4	70.3±4.1	42.1±3	65.5±1.5	35.9±0.83	25±2.3
LNCaP	96.8±5.4	71.7±2.2	45.1±5.0	91.5±6.3	66.6±5.7	38.5±3.8	58.4±2.9	39±1.9	30.7±2
PC3	96.4±5.0	80.1±4.6	73.2±4.1	89.9±3.6	70.7±2.8	28.5±3.4	61.7±2.1	46.9±1.4	31.5±3.7
PC12	91.1±6.5	68.5±6.8	40.3±4.4	94.4±5	62.5±4.6	30.7±3.8	50.4±3.6	40.8±1.8	22.1±1.1
MCF-7	94.8±6.1	77.5±5.1	47.1±4.2	81.3±4.1	55.5±3.4	25.2±2.1	51.6±3.2	35.7±2.5	19.1±1.4
MOLT-4	88.6±3.6	57.5±4.0	42.8±3	70.6±2.8	43.1±1.9	21.6±1.4	11.5±0.5	10±0.37	2.1±0.9
U266B1	95.1±4.9	53.8±4.6	33.8±4.7	68.5±2.3	37.2±2	20.4±2.1	15.9±0.8	4.8±0.72	5.5±0.38
Raji	85.6±4.1	68.1±2.8	29.4±4.6	60.6±3.6	30.3±2.4	14.6±3.3	5.5±0.4	1.3±0.25	0.18±0.09
CHO	97.7±5.2	74.4±4.1	70.5±5.2	97.4±4.4	64.6±2.8	21.9±3.5	57.8±3.9	39.2±2.7	20.7±3.7

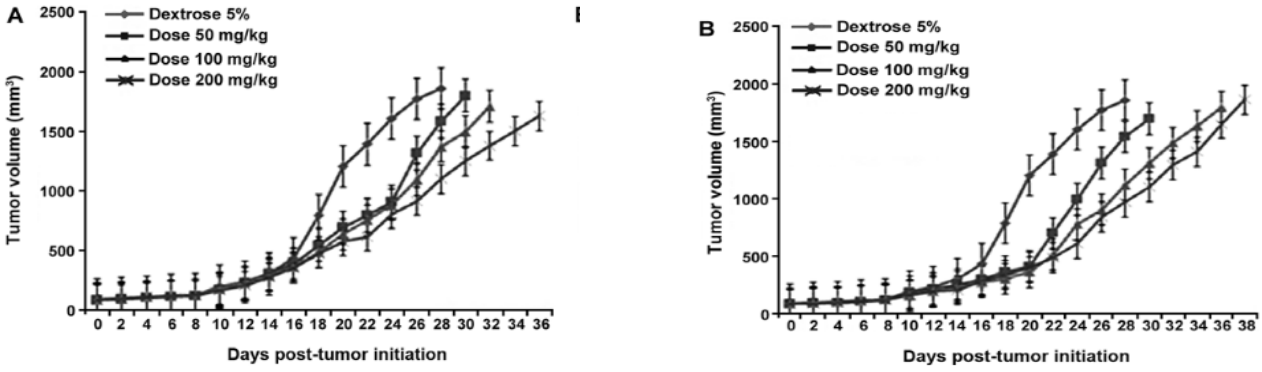

Figure (8). The relationship between tumor volume and days after tumor initiation. Whereas (A) CT26 and (B) MCF 7 cell lines were treated with (50, 100 and 200 mg/kg) doses of quercetin for several days (Hashemzael et al., 2017).

Discussions

The effect and activity of quercetin have been studied as an anti-cancer drug (Niu et al., 2011), (Mukherjee et al., 2019). In the vitro, different concentrations of quercetin were applied to different cancer cells. After hours of taking the quercetin doses, the inhibition effect of some cancer cell lines was very high, as shown by the IC50 values calculated in Table (2). The IC50 scale was used to describe the relationship between a series of drug dose concentrations and the response to the effectiveness of these concentrations in inhibiting 50% of the activity of cancer cell lines (it means inhibiting half of the total response to get 50% of the effect), and it was found that quercetin inhibited the activity of cancer cell lines. (Hashemzael et al., 2017)

In Figure 6, the study also showed apoptosis of the following: CT 26, LNCaP, MOLT 4 and Raji cell types by used flow cytometry Annexin V/PI and compared with control group (Hashemzael et al., 2017). Also, in the vivo, models of mice with positive breast cancer of estrogen receptor "MCF 7" and CT 26 were treated with different doses of the quercetin at concentrations of about (50, 100, and 200 mg / kg) for several days. The results from using quercetin showed in Figure (8) that the tumor size for each of these mice became small after receiving the required doses. (Hashemzael et al., 2017)

Both in vivo and in vitro, quercetin was also successful in suppressing the growth of human LM3 hepatocellular carcinoma by collecting the LM3 cell line treated with different doses of quercetin at different time intervals resulting in apoptosis at $P < 0.05$. (Wu et al. 2019). Treatment with 160 μM quercetin for 24 h suppressed the viability of oral squamous cell carcinoma "OSCC" cells by inducing cell cycle arrest on "OSCC" cell lines using MTT assay, flow cytometry and blot analysis ($p < 0.01$), "whereas treatment with low concentrations10–40 μM) reduced cell viability slightly" ($p < 0.05$) compared to the control. (Kim et al, 2020)

Conclusions

Most of the chemical treatments for cancer have annoying side effects, so nature has been taken care of in terms of eating vegetables, fruits and seeds because of their benefits on the human body. The balance of food in your diet is important when you are trying to eat healthy food. Eating these healthy things would enhance the body's immunity and provide it with the necessary antioxidants, which target the elimination of increasing free radicals. In order to provide the best ways to treat cancer and help cancer patients, a review was discussed about the causes of cancer and quercetin extract and its applications in vitro and in vivo rather than many methods used in treatment such as chemotherapy. In vitro, the inhibition effect of all cancer cell lines was very high, as the study showed apoptosis by using Annexin V/PI flow cytometer and comparing it with the control group. The results of using quercetin showed that the tumor size for each of these live models was small after receiving the required doses.

Acknowledgements

The author would like to acknowledge those who always encourage and give their guidance, mom and dad.

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