

The Effect of quercetin on induction of apoptosis and cell proliferation in experimental rat colon carcinoma

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Abstract

Colon cancer is the fourth cause of mortality in the world and the second most common cancer in Europe (1). About 8% of overall mortality rate caused by cancer is caused by colon carcinoma and the highest spread of colon cancer is reported in Asia and Eastern Europe (2). Among cancer preventive factors by chemical mechanisms reducing risk of cancer, Phytochemicals can be the most effective factors. Among the Phytochemicals, one can refer to quercetin. Quercetin is the second flavonoid in plant species, which is available in fruits and the majority of vegetables (36) and can be considered as an important cancer prevention compound. The cancer preventive effects of the compound can be attributed to antioxidant activity of quercetin, inhibition of carcinogen activating enzymes, regulation of intercellular signal transfer paths and interaction of quercetin with receptors and other proteins (37). According to studies, quercetin can inhibit sulfate and hence, it can increase biofeedback and treatment competency through inhibition of sulfate.

Key words: quercetin, apoptosis induction, cell proliferation, rat colon

Introduction

Any kind of abnormal cell growth causing natural change or mutation in a cell is called cancer. Cancer cells are not limited to common limitations in cell proliferation applied by the host. However, proliferation does not necessarily refer to cancer. Abnormal cell growth can be divided into two groups of neoplastic and non-neoplastic classes.

Non-neoplastic growth patterns

4 patterns of non-neoplastic growth are as follows:

1) Hypertrophy: it means increased cell size, which happens mainly as a result of increased work load or hormone stimulation.

2) Hyperplasia: refers to reversible increase in the number of cells in a special type of tissue causing increased tissue mass.

Hyperplasia is considered usually as a natural physiologic response in the case of fast growth (like pregnancy and maturity)

3) Metaplasia: a type of mature cell is replaced by another cell type, in which no tissue is usually observed (e.g. replacement of tubular cell instead of squamous cell). In case of removal of factor, the process can be reversible; although it may be changed into dysplasia if the stimulation is continued. Metaplasia may be created because of inflammation, vitamin deficiency, irritation and various chemicals. One of the most common areas of metaplasia is in the cervix.

4) Displasia: in this mode, normal immature cells change in terms of size, formation or organization or a mature cell is replaced with an immature cell. Displasia stimulants have usually external sources (like radiation, toxic chemicals and chronic irritation). Displasia may return in cases of elimination of irritant.

Colon Cancer

Colon cancer appears in the form of some glands in inner wall of colon. Colon cancer has considerably increased in Iran over the past 3 decades. According to the annual report of the National Cancer Institute of Iran, colon cancer is currently the second most common cancer in Iran. Early diagnosis of colon cancer over the years has played a considerable role in survival, since chemical medicines can prevent seamless cell proliferation in certain tissues of body organs and induction of apoptosis in tumor cells. However, one of the most common problems in this field is that the recent treatment methods for advanced colon cancer can impose damaging effects on the health system and the patient. Effectiveness of treatment, even with development of new anti-cancer drugs, is not satisfactory and the American Society of Clinical Oncology (ASCO) has emphasized necessity for a new approach for treatment of cancer. The main objective of colon cancer treatment should be purposeful use of cancer prevention materials with chemical mechanism. Among cancer preventing factors with chemical mechanism causing reduction of risk of cancer, phytochemicals are the most effective materials. Phytochemicals are non-nutritious herbal compounds with disease prevention properties with anti-cancer effects. One of these compounds is natural material (3, 4, and 5-trihydroxytrans-acetilin) with abundant biologic effects to prevent and treat cancer. Another phytochemical is quercetin. Quercetin is the second flavonoid in plant species available in fruits and the majority of vegetables. Phytochemicals can have preventive effects against cancer from the beginning of creation of tumor to all factors affecting cancer (cell proliferation, apoptosis, inflammation, genome stability). The main mechanism is expressing preventive function of cancer with chemical mechanism of natural elements of edible plants including adjustment in expression of cell proliferation regulating genes, adjustment in differentiation, adjustment in apoptosis and prevention of angiogenesis and metastasis. Moreover, quercetin can inhibit sulfate and hence, it can enhance biofeedback and as a result, treatment competency through inhibition of sulfate. It seems that among all mentioned mechanisms, apoptosis induction and cell proliferation inhibition can be the most important mechanisms affecting anti-cancer activity of phytochemicals, since can cause induction of mechanisms in line with apoptosis similar to drugs for cancer treatment.

Therefore, treatment of advanced colon cancer steps can be considered as a challenge. Hence, analysis of the effect of quercetin in induction of apoptosis, cell proliferation level and colon cell abnormalities can be important. Because of conflicting reports on the effectiveness of quercetin in different steps of cancer and lack of studies investigating

the two compounds simultaneously in colon cancer in vivo; the present study tends to evaluate their combined effect, so that the results obtained from the study can be generalized to human studies.

Quercetin

Quercetin is a polyphenyl product in plants. Quercetin is one of the most important and available compounds of flavonoid strain, since it has the highest antioxidant properties among other flavonoids and is even 6 times stronger than vitamin C. Quercetin is available in vegetables, fruits, onion, apple, red grape, citrus fruits, Brussel sprouts and tomatoes, Green tea and dark chocolate (11-13). The relevant studies show protective effects of quercetin against DNA factors, liver, heart, kidneys and neurons (14). Moreover, the compound has pathogenic effects (16) and also has anti-cancer, antiviral, antimicrobial, anti-allergic, anti-hypertension and protective property against cataract (17). Quercetin also has antioxidant and anti-inflammation properties and can cause serum level protection (13), Glutathione and reduced serum level of malondialdehyde and decrease of nitric oxide metabolism and formation of superoxide, as well as decrease of release of oxidizing and inflammatory mediators (19). The compound has protective effect against oxidative damage and with decrease in oxidative damage in alanine transferase of liver, prevents increase in alkaline phosphatase (ALP) and Aspartate transferase (GPT) (20). There is no comprehensive information in regard to the effect of quercetin on liver and kidney damage caused by Methotrexate and previous studies have investigated limited parameters (21). Therefore, the study also investigates the effect of quercetin on removal of the toxic effects of Methotrexate on liver and kidney tissue and antioxidant enzymes of liver and kidney.

Necrosis and Apoptosis

There are two general paths for death of a cell: necrosis and apoptosis.

In the definition of necrosis, it should be mentioned that the cell is damaged as a result of external factors like toxins, pollution, infection and cessation of blood supply. The damaged tissue is usually inflamed and creates abundant problems.

However, apoptosis is an intercellular process: apoptosis or programmed cell death is a kind of cell suicide. The main advantage of apoptosis compared to necrosis is that apoptosis is a predictable and controllable process in most cases and can be removed easily by macrophages.

Apoptosis

In the Greek language, apoptosis means fall of leaves of trees or petal fall in plants. The process taken in multicellular animals is a vital process in body cells. In an adult person, daily about 50-70 billion cells fall in apoptosis and 20-30 million cells fall in apoptosis in a 8-14 year old child.

When 1 cell has to suicide by apoptosis inductor, proteins called caspase come into action. Caspase proteins can affect the process of DNase production in cell and the DNase enzymes cause cell wrinkle through intercellular DNA destruction (cell contraction). In the next step, signals are given to Phagocyte proteins and hence, the apoptosis process is completed with Epofototic cell phagocytosis. Apoptosis is a vital process during the evolution of multicellular animals. For example, destruction of the curtains between fingers and toes are taken in fetal steps during apoptosis. Moreover, during the evolution of the brain, more cells are produced than required. Therefore, those without production of synapse joints are destroyed during apoptosis. Apoptosis is also essential during the menstrual process.

The studies on apoptosis were developed in the years after 1990; that is, the time that its role in different diseases was specified. It is very important that apoptosis is not always a perfect procedure. Sometimes, cells enter into the apoptosis path wrongly and the wrong procedure can be because of presence of apoptosis irritants. For example, when a cell is exposed to free radicals or directed radiations or is affected by stress, apoptosis may happen. Sometimes, it happens reversely: cells under apoptosis don't enter the apoptosis path (like cancer).

Scientists try to control apoptosis procedure of cells and control the type of apoptosis cell to treat diseases such as cancer and AIDS, Parkinson and Alzheimer. Today, treatment of cancer is being undertaken using chemotherapy or radiotherapy based on target cell apoptosis stimulation.

Discussion and literature review

According to statistics of the World Health Organization (WHO), colon cancer is being diagnosed annually in 1 million people of the world (3). Colon cancer has considerably increased over the past 3 decades in Iran and according to the annual report of National Cancer Registry of Iran; it is the second most common cancer in Iran in terms of spread (4). Colorectal cancer spread in Iran is equal to 8 out of 100,000 people (5). It has been estimated that every year in Iran, 3,641 new cases of cancer happen and 2,262 people die annually as a result of colon cancer (6). Spread of this cancer in Iranian population is equal to 22% (4). According to relevant studies in Iran in the field of age distribution of colorectal cancer, this cancer is happening in a younger population in Iran compared to western countries (3).

Initial diagnosis of colon cancer over the years has played key role in survival, since chemotherapy drugs can prevent excessive cell proliferation in certain tissues of body organs and apoptosis induction in tumor cells (7). However, one of the most important problems is that the recent treatment methods for advanced colon steps can impose damaging effects on the health system and on the patients (8).

The common method of cancer chemotherapy is that the effective substance is entered into the body and the substance affects all cells and tissues in addition to cancerous cells and more importantly, it can damage adjacent body tissues (7).

Effectiveness of treatment is not satisfying even using new anti-cancer drugs and the American Society of Clinical Oncology (ASCO) emphasizes the necessity of finding a new approach for cancer treatment. The main objective in treatment of colon cancer should be purposeful use of cancer chemoprevention substances with a chemical mechanism (9).

Sliddique et al (10) studied the effectiveness in cancer through prescription of one or more natural or synthetic agents to prevent cancer relapse or slow down the disease progress as cancer chemoprevention.

The main purpose of cancer chemoprevention is to identify the natural components of ingestible plants preventing growth or metastasis of cells through interference of intercellular paths of cancer cells (9).

The main mechanism presenting the cancer chemoprevention is the natural components of ingestible plants including modulation in cell proliferation regulating gene expression, modulation in differentiation, modulation in apoptosis and angiogenesis and cessation of metastasis (11).

The contents and components of natural elements of different foods can be the most reliable compounds to monitor cancer, since their effects have been studied over the years in the field of relevant accidents of cell process and pleiotropic and non-toxicity for normal cells. Food material can be considered as one of the environmental factors responsible for 20-30% of colon cancer cases, since food materials can be considered as microenvironment determinant factors of colon cancer cells and the interactions between cancer cells and microenvironment around cell can also affect tumor growth (2).

Among chemoprevention factors decreasing risk of cancer, phytochemicals can be the most important factors (12-15). The majority of studies conducted over the 3 decades have introduced the materials extracted from plants as factor suppressing or creating delay in progression of types of cancers. The results are consistent with the findings of epidemiologic studies showing that using fruits and green and yellow vegetables can reduce cancer and mortality in colon, breast, prostate, esophagus and bladder cancers (14, 16, 17). Phytochemicals are non-nutritious herbal compounds with anti-cancer effects (18).

According to the book "Fundamentals of Cancer Prevention", cancer prevention happens in 3 steps: the first step (mainly prevention of carcinogens), second step (identification and elimination of benign lesions) and third step (prevention of cancer relapse, tumor progress)

Phytochemicals can have preventive effects in 3 steps against cancer from the beginning of tumor creation to all effective factors in cancer (cell proliferation, apoptosis, inflammation, genome stability) (11, 18).

As 2 main hallmarks of cancer cells are abnormal cell proliferation and resistance to apoptosis, identification of natural herbal edible components can be the most reliable compound (because of non-toxicity) to monitor cancer treatment (19, 20).

One of these compounds can be a natural substance (3, 4, and 5-trihydroxytrans-acetilin) with abundant biologic effects to prevent and treat cancer. A phytoalexin compound or antibiotic is the plant produced in the plant abundantly in response to environmental stress and pathologic attack and acts therefore as a natural prevention factor of cell proliferation (21, 22). The compound is produced from attachment of cinnamoyl radicals (two) (23). It has been identified in more than 70 plant species including grape, berries, plums, peanuts and pine (21). Over the decades, it has been found that it has wide range of pharmacologic properties. It seems that additional biochemical and molecular activities in this substance can cause effects in cancer and pre-cancer cells (11, 22).

The information has led to take numerous basic animal studies to investigate the potential effects as a cancer chemopreventor substance. Moreover, it also imitates calorie limitation and promotes health and interferes in the ageing process (22).

It has been reported that it can inhibit accumulation of Platelets and LDL Oxidation, Nitric Oxide Synthesis and vein expansion in vivo (19) and inhibition of proliferation of smooth muscle cells through reduction of Cyclin A gene expression (24). Moreover, the compound can suppress cancer cell growth (in vitro) (1) and reduction of tumor growth in animal models (13). Recent studies have shown that it can cause apoptosis induction in cancer cells through increasing P53 gene expression and reduction of Bcl-2 gene expression (26).

The proposed mechanisms for anti-cancer effects include cell death induction through Fas level regulation in cells producing extracellular apoptosis (27-29), increase in caspase activity (30, 31), decreased inflammation through suppression of gene regulation products with NFKB (31), decrease in production of proteins associated with cell cycle (Expression of cyquilin-dependent kinases E, Cdk4, cyquilin D1) (31, 32), increase in SIRT1 expression (gene relevant to decrease in expression of surviving, cell life cycle and slowing down the ageing process) (34) and wingless inhibition Wnt (35).

Another phytochemical is quercetin. Quercetin is the second obvious flavonoid in plant species and is available in fruits and the majority of vegetables (36) and can be considered as a cancer prevention compound. Cancer prevention effects of the compound can be attributed to antioxidant activity of quercetin, inhibition of carcinogen activating enzymes, regulation of intracellular signal

transfer and interaction of quercetin with receptors and other proteins (37).

Among proposed anti-cancer molecular mechanisms, direct effect of quercetin on reduction of Cyp1A7 activity in colon cancer (playing a role in activation of carcinogens) (37) can be referred to. Also, one can refer to the effect on estrogen and inhibitory effects on expression and yield of androgenic receptors (a similar activity of phytoestrogens) (38), induction of apoptosis through the Mitochondrial path (activation of caspase 3 and 9), decrease in Bcl-xs/Bcl-xl ratio and increase in Bax (39), effect on DNA failure, Poly (ADP-ribose) Polymerase (PARP) failure and increase in Bax and effect on Bcl-2 level (anti-apoptosis) (39), decrease in synthesis of inflammatory cytokines and iNOS gene expression (40).

Quercetin and both polyphenoles are available in red grapes. It has been demonstrated that simultaneous use of quercetin and polyphenole can cause reduction of restinosis level (probably through inhibition of smooth muscle cell proliferation). Therefore, using a combination of quercetin and polyphenole has high potential in cancer control (41).

Moreover, quercetin can cause inhibition of sulfate process and increase biofeedback and ultimately, treatment competency through inhibition of sulfate. However, further studies are needed in this field (22).

It seems that among all mentioned mechanisms, apoptosis induction and cell proliferation inhibition can be the most important underlying mechanisms affecting anti-cancer activity of phytochemicals, since these factors can cause apoptosis induction in the field of cancer treatment (42). Apoptosis or cell planned death is a normal regulated process of suicide enabling living things to preserve cells and eliminate unwanted cells threatening their survival. It seems that apoptosis is useful physiologically, since cells with apoptosis are removed by Phagocytosis before losing cell plasma membrane permeability. In this mode, cells with apoptosis induction in macrophages (especially phagosomes) are declined without damaging adjacent tissues (43).

Apoptosis is regulated differently from distance. In the extracellular path, it happens through activating apoptosis death receptors and through changing permeability of mitochondrial membrane. Defect in apoptosis plays a key role in formation of tumors and creation of neoplasia and failure of its order can cause resistance to chemotherapy and radiotherapy and can ultimately increase metastasis. Finally, both paths activate ultimately a family of proteases called caspases (CysteinyI Asparatete Specihi Proteinase) (44). Caspases are members of the Cysteine protease family and play a key role in beginning and implementation phase of apoptosis.

More than 100 types of Substrates caspases have been identified to date and new substrates are regularly added to this list, which can be catalyzed by caspases. Substrates of the proteases include Lamine, Actin, Endonucleases,

DNA repairing proteins and finally transcription factors. Activation of caspases is mostly specified to apoptosis and determination of activity of caspases can be used to differentiate necrosis and apoptosis (45).

In addition to apoptosis induction, cell cycle cessation is a hopeful strategy for cancer prevention. Cell cycle includes 4 steps respectively including G1, S, G2 and M (46). Abundant factors and proteins in various positive or negative controlling points can regulate the cycle carefully. In case of the existence of ideal factors (e.g. quercetin), cancer cells in G1 are induced by apoptosis, otherwise; the process enters to phase S with activity of other passing mechanisms. With continuity of the ideal factors (like quercetin), with increase in cancer cell treatment time, apoptosis is induced. Cell proliferation plays a key role in multiple carcinogenic steps with genetic variations. Therefore, cell proliferation control is important for cancer prevention (46). Quercetin and other flavonoids can inhibit proliferation of colon cancer cells (1) and stomach cancer cells (43).

In addition to bromodeoxyuridine method Brdu in DNA and analysis of cell cycle protein analysis, the number of Argyrophilic Nucleolar Organiser Regions (AgNoRs) is used to evaluate cell proliferation in many organs such as colon mucosa (10). Recent studies have shown that using quercetin can lead to considerable decrease in number of tumors in the colon and can also prevent tumor creation in the colon of Min rats (13, 46, 47). It should be mentioned that cell proliferation changes in epithelial colon cell proliferation can increase risk of colon cancer.

Quercetin (48) and (47) can cause decrease in D1 and D2 cyclin gene expression, which can directly play a role in progress of the cell cycle and it has been also demonstrated that the substance has anti-cell proliferation effects and can make pauses in the cell cycle in vitro (20).

Analysis of the effect of quercetin in apoptosis induction, cell proliferation level and colon cell abnormalities is very important. Because of conflicting reports on effectiveness of quercetin in different steps of cancer (22, 24) and lack of studies investigating simultaneous effect of the two compounds on colon cancer in vivo; the present study tends to investigate the simultaneous effect of the two compounds, so that positive results obtained from the study can be generalized to human studies.

Positive effects created in inhibition of toxicity of Methotrexate can be attributed to antioxidant properties of quercetin, which can increase antioxidant capacity and can also decrease oxidative stress in cells. Antioxidant capacity of plasma in the second group was significantly reduced compared to the control group. However, antioxidant capacity of plasma was significantly increased in the group under treatment with quercetin. At the same time, it is decreased with serum tissue and kidney tissue of MDA and FRAP has been also increased. The process can be attributed to the presence of quercetin antioxidant (13). Moreover, in a study, Abdolvahab et al studied the effect of spinaciaoleracea on Methotrexate liver toxicity in rats and

showed that decrease in ALT and MDA Methotrexate can increase glutathione. Treatment with extract of this plant can lead to Hepatobiliary disorders. Significant decrease in MDA was observed and the effect was associated with the antioxidant property of quercetin in spinaciaoleracea (36), which is consistent with histological results obtained from kidney tissue in this study. Moreover, in this study, the results of histopathologic studies of kidney and liver are consistent with biochemical tissues and confirm the findings (2, 3 and 4) (Figure 1). In this study, the results showed presence of severe lymphatic arthritis in kidney and liver tissue of the test group without treatment and getting just Methotrexate (Figures 3 and 4). However, prescription of quercetin can decrease lymphatic arthritis and generally, decrease damage. In the study on liver and kidney, the effect of quercetin, Rutin and allopurinol on uric acid level and kidney dysfunction caused by fructose intake in rats showed that the materials can decrease uric acid, urea, creatinine and decrease inflammatory cell infiltration in Crossbone tissue of the rat's kidney (37) and the findings are consistent with results of this study. However, it was observed in this study that serum concentration of urea in the second group (test group without treatment) showed significant decrease compared to the group getting silymarin (group 4) and had a liver protection antioxidant (26) and the effect was probably caused by liver damage by Methotrexate and as a result, decrease in urea synthesis in liver urea cycle, since in the group under treatment with silymarin, serum concentration of urea was almost close to serum level of the control group. In general, in this regard, one can refer to the studies conducted on quercetin, which showed that quercetin has anti-inflammatory, and antioxidant properties and also provides strong protection (15, 16, 18). Kidney and liver superoxide is an enzyme available in cytoplasm Cell Dismutase (SOD) and is also one of the defensive lines against free radicals and can protect tissues against active hydroxyl radicals (3). Quercetin is a penta-hydroxyl flavonoid in fruits and vegetables.

Foods like green and black tea, apple and onion are rich in quercetin with antioxidant and anti-inflammatory, antiviral and anti-cancer properties for a wide range of diseases (12, 14). It also has anti-cancer properties in ovarian, colon, intestine and breast cancers. Studies have shown that quercetin has anti-proliferation effect in cancer cells during inhibition of PKB/PI3K/Akt path (13). Quercetin can be used to treat AIDS, malaria, cardiovascular disease as well as cancer (10) and can induce apoptosis in breast cancer cells and prevent their growth (16, 19). Moreover, quercetin can inhibit Angiogenesis in tamoxifen-resistant Breast cancer (17). As different materials show their effect through affecting cell proliferation, to use the two materials, the effective dose and toxicity of the materials should be obtained in breast cancer cells above all. To this end, the amount of IC50 is determined as required dose to inhibit growth of 94% of cancer cells.

Conclusion

Curcuma longa is a type of plant from ginger and Yellow Chubs strain used for thousands of year for treatment of diseases such as cold, fever, skin diseases, liver diseases and stomachache and it is not toxic even in high doses. Curcumin (Di Ferrouilly Methane) is a Polyphenylene from Ariel Heptanoids strain and quercetin is also one of the flavonoids from flavonole strain and form the main compounds of yellow chub. The two substances have been considered as an effective compound to treat cancer. Some studies have confirmed significant correlation between diet of the two substances and reduced level of cardiovascular diseases and cancer and it can be used to make antibacterial, antiviral, anti-fungal and anti-tumor medicines. The two natural substances have anti-cancer properties. Various studies have been conducted to determine the treatment dose and to determine their IC50 on types of cancer cells (1-3). In this study, the percentage of survival and IC50 on breast cancer cells of 4T1 rats are determined. The results show that survival of cells in the control group has shown the highest level and the level is considered to be 100%. However, after 20 minutes and 8 hours contact with curcumin, the survival percent is reduced and the reduction has only depended on concentration. Regarding quercetin, at two times of 20 and 08 hours, it was found that reduction of survival percentage depended on both factors of concentration and time and with increase in both factors, the cell survival percent is reduced significantly. Regarding curcumin, the amount of IC50 at the time of 20 hours is equal to $10.8 \pm 4.0 \mu\text{g/ml}$ and at 08 hours, it is equal to $21 \pm 4.3 \mu\text{g/ml}$. Regarding quercetin, the amount of IC50 was equal to $21.7 \pm 4.7 \mu\text{g/ml}$ at 20 hours and equal to $18.2 \pm 4.09 \mu\text{g/ml}$ at 08 hours.

Feng Zhang et al investigated the effect of quercetin on HEN1 cells and showed that it can decrease cell

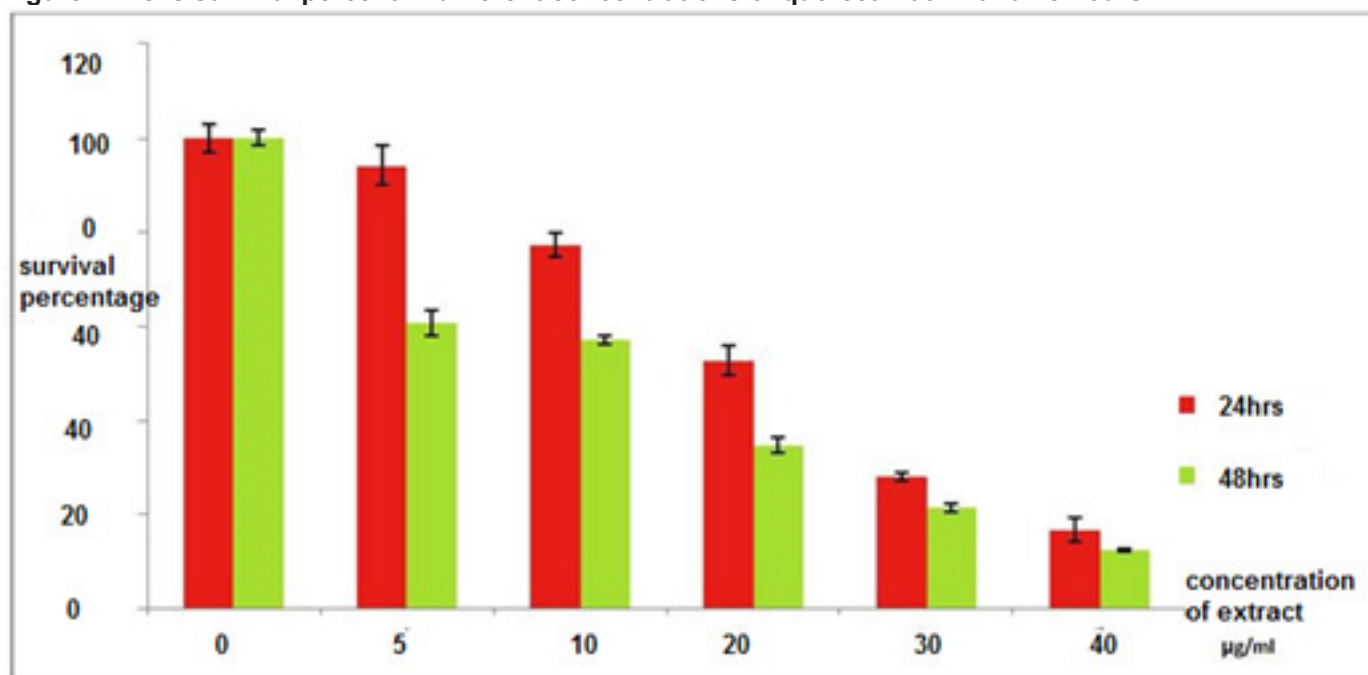
proliferation and cell lifetime significantly (22). Vidya et al studied the toxicity of quercetin on Hela cells and mentioned that with increase in concentration and time, the lifetime of cells was decreased and IC50 was mentioned at $84 \mu\text{mol}$ (23). Singhal determined IC50 at first to analyze the effect of quercetin on activity of enzymes in breast cancer cells MDA-MB-435 and showed that with increase in concentration, the inhibition percent is increased (20). As the IC50 curcumin and quercetin level is different for different cancer cells and because of high spread of breast cancer in Iran and closeness of 4T1 rat cell line to step 0 of human breast cancer, this study has used this cell line. In this study, toxicity of the two medicines on 4T1 cell strain is studied using MTT method and IC50 level is also determined for each of them. Moreover, the results obtained from this study showed that the percentage of survival for curcumin at 20 and 08 hours showed no significant difference; although for quercetin, with increase in time, the percentage is decreased significantly, so that the survival percentage at 20 hours is more than 08 hours.

The results obtained from this study showed that the survival of cells is dependent on concentration of curcumin and quercetin and the incubation time. With increase in solution concentration, the toxicity is increased and survival of cells at 08 hours has been decreased compared to at 20 hours.

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Figure 1: Cells survival percent in different concentrations of quercetin at 24 and 48 hours



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