Side effects of Antioxidants over Cancer Therapy

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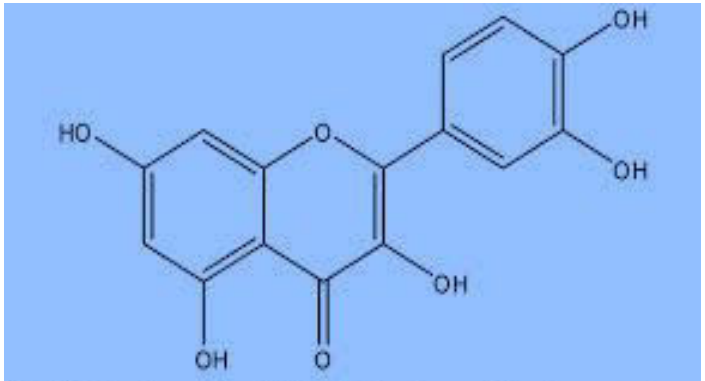
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Cancer: One of the biggest life threatened problem

* Cancer is occur after DNA damage of cancer precursor genes (oncogene) such as p53, Ras and BCL genes.
* Radiation, Toxic metals, Viruses, and excessive metabolic free radical generation may lead to DNA damage and cancer.
* Cancer is second life threatened illness after cardiovascular diseases on the world.
* So, Cancer patients intends to use traditional medicine. Especially, uses of therapeutic herbs is very popular,
* At this point Uncontrolled uses of herbs and fruit extracts are really safe?

Antioxidants and Quercetin as free Radical scavenger

* Quercetin is one of the most abundant dietary flavonoids. It can be found in apples, black, green and buckwheat tea, onions, raspberries, red grapes, cherries, citrus fruits as well as in some well known medical plants (ginkgo biloba, cranberries and St John's wort) (Hertog and Hollman, 1996; Terao, 2009).
* In previous *in vitro* studies has shown that quercetin acts as an antioxidant and anti-inflammatory agent and that it has potent anticarcinogenic properties as apoptosis inductor .
* Apoptosis: Programmed cell death,



2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one
(**quercetin**, flavonol)

Free radical scavengers and Hydrogen peroxide

* In the normal conditions, herbal sourced antioxidant intakes could be beneficial for cancer prevention (Gerhauser, 2008; Murakami et al., 2008), coronary artery disease prevention (Siu, 2010) and moderation of aging process.

* **But what will happen if cancer patient takes concentrated herbal sourced antioxidants such as quercetin which is abundantly present in many herbs, fruits and grains during cancer therapy. Will it facilitate cancer therapy or not?**

* To examine that, a simple study was designed. Erythroleukemia cell line (K562) was induced to apoptosis by hydrogen peroxide and effect of quercetin over the apoptosis and viability of erythroleukemia cells was observed with flow cytometer.

* Note: Hydrogen peroxide produces during cancer radiotherapy and chemotherapy, and it is one of the important keys to start apoptosis processes in the cell companents

Reactive oxygen species (ROS)

* Reactive oxygen species (ROS) are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. These side products can damage many biological molecules; they can change protein functions, damage DNA material and cause lipid peroxidation of cell membranes (Kulbacka et al., 2009).

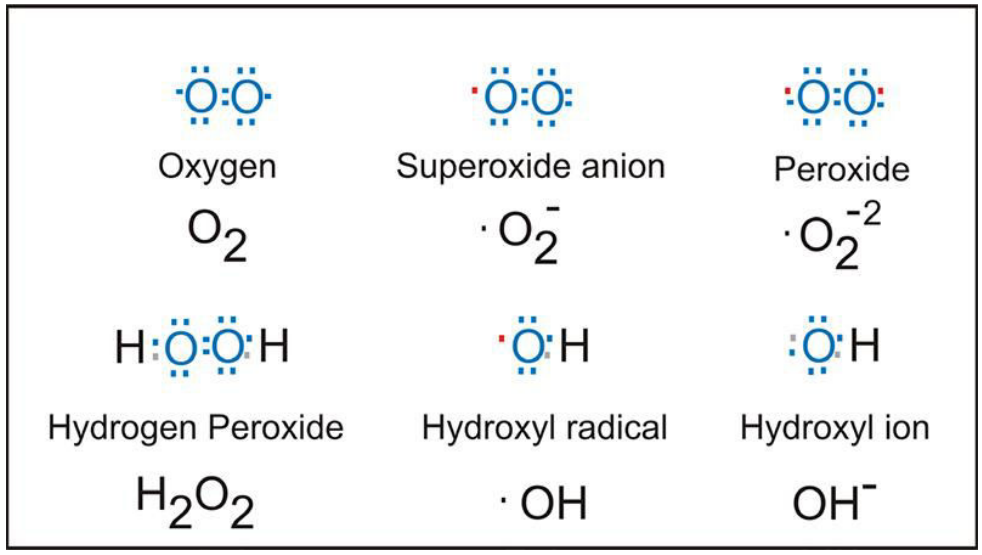
* In general, the reducing environment inside cells helps to prevent free radical mediated damage. This reducing environment is maintained by the action of antioxidant enzymes and substances, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, vitamins C and E (Bayir, 2005; Schafer et al., 2001).

* Due to DNA damagin effect, Reactive oxygen species are key companents to destroy cancer cells via radiotherapy and chemotherapy

Reactive oxygen species (ROS)

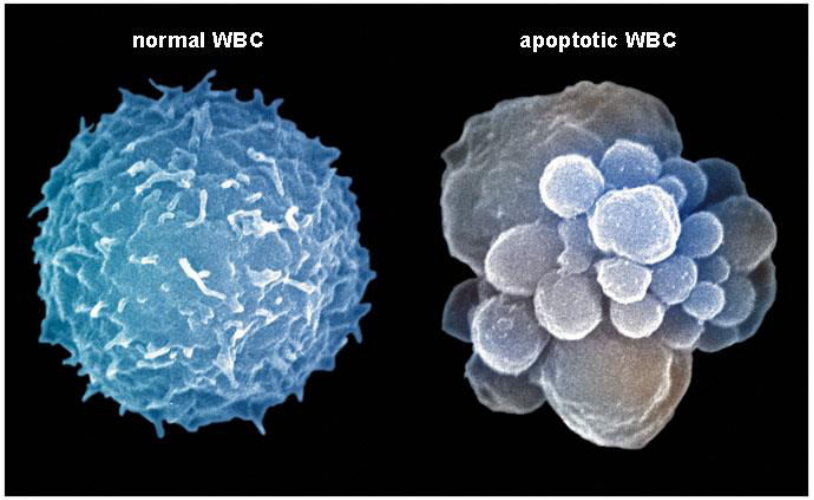
* Excessive production of ROS may lead to programmed cell death and may accelerate the process of aging (Chamond et al., 1999).

* For a long time, reactive oxygen species (ROS) have been considered harmful mediators of inflammation owing to their highly reactive nature. However, there are an increasing number of findings suggesting that ROS can play role in anti-inflammatory and prevent autoimmune responses

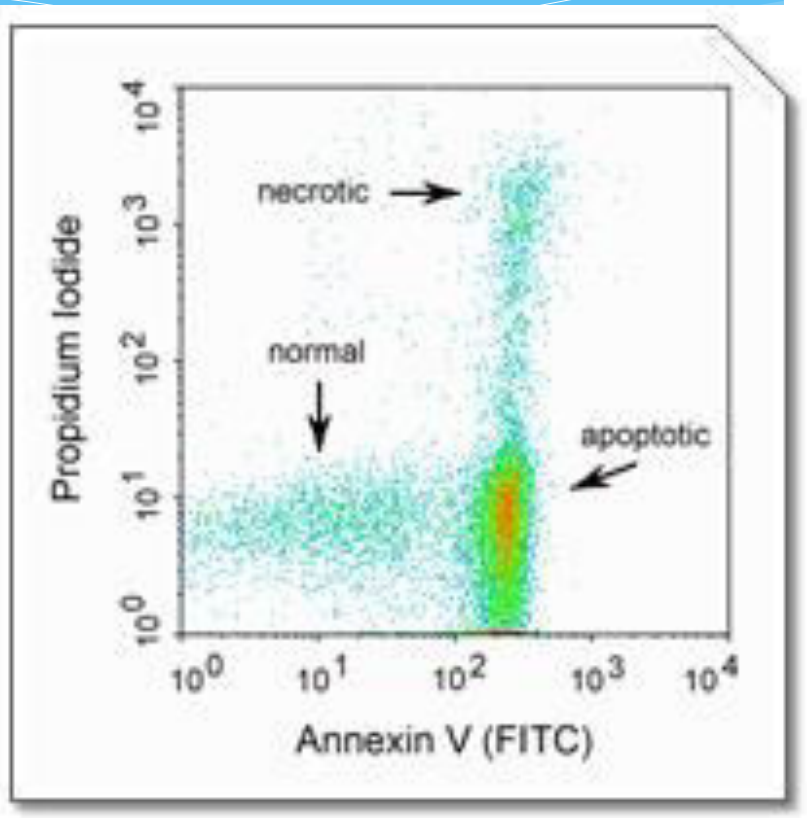


Material and Methods

* ***Apoptosis induction by quercetin***
* Effect of quercetin (Sigma-Aldrich, St. Louis, MO, USA) on the erythroleukemia cell viability was evaluated with different Quercetin concentration (5-500 µM) through 3 hours with Flow cytometer (Becton-Dickinson, San Jose, CA, USA). Toxic dose of Quercetin concentrations (100- 500 µM) was evaluated through 24 hours.
* ***Apoptosis induction by hydrogen peroxide***
* erythroleukemia cells (K562) were induced to apoptosis by the hydrogen peroxide (H_2O_2 Riedel de Haen, D3016, Seelzel, Germany) in different concentration (150, 300, 600 µM) and protective effect of Quercetin (100 µM) was analyzed through 24 hours. For this aim, protective dose of Quercetin (100 µM) was added on to K562 cell (10^6 cells in 10 mL RPMI 1640, 10% FCS, penicillin/ streptomycin), Then hydrogen peroxide were added in to RPMI cell culture medium.



* ***FITC-Annexin V staining assay for apoptosis detection***
* After induction with H_2O_2 and Quercetin, K562 cells were washed twice with PBS and suspended in 1x binding buffer (10 mM HEPES, 140 mM NaCl, and 5 mM $CaCl_2$ at pH 7.4) at a concentration of approx 10^5 cells/ml. Five µl of FITC-Annexin V and 10 µl of propidium iodide (Sigma, A2214, 1;100) was added to both control and induced cell suspension from the 50 µg/ml stock. After incubation at room temperature for 10 minutes at dark, the fluorescence of the cells was determined immediately with a flow cytometer (FACS Calibur, Becton-Dickinson, San Jose, CA, USA).
* ***Statistical analysis***
* **Measurements were repeated more than 3 times and student t-test was used for statistical analysis.**



Results

* For the time-course and dose-response experiments, K562 erythroleukemia cells were treated with different concentration of quercetin (5, 10, 20, 40, 80, 100, 200, 300, 400 and 500 µM of quercetin) for 3 hours. Figure 1 shows that quercetin (In the 5-200 µM doses for 3 hours) caused slight increase of K562 cells viability (number of alive cells) (Figure 1) but high doses of quercetin (over than 200 µM) caused to increase of apoptotic cell death ($p<0.01$).

* Some of previosly studies were indicate to apoptotic effect of quercetin even if low quercetin doses such as 5 to 100 µM in the literature !

* **As we expected, Contrary to the literature , quercetin has protective potential over the cells in the normal environment**

* For second experiment K562 cells were treated with 100, 200, 300, 400 and 500 µM of quercetin for 24 h. Toxic doses of quercetin (doses higher than 100 µM, 24 hours) resulted with decrease of K562 cell viability as expected ($p<0.01$) (Figure 2).

* Thus, 50-100 µM of quercetin was determined as the protective quercetin dose range for the cells

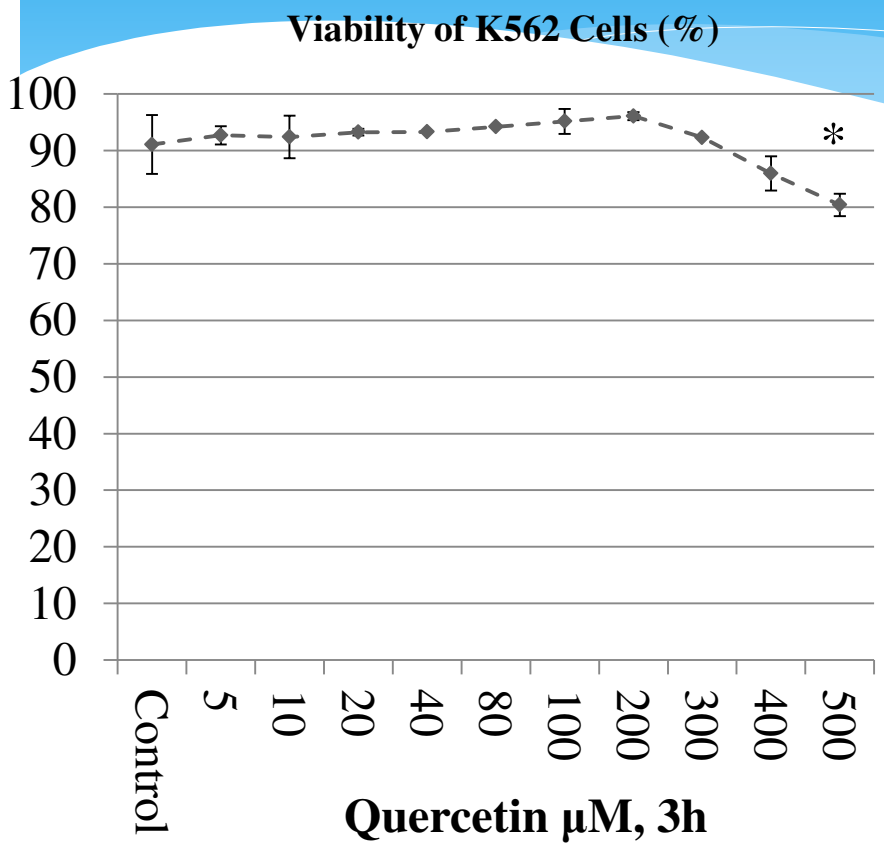


Figure 1. Effect of Quercetin Over the Viability of K562 Cells for 3 Hours. Non-apoptotic cell rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. *p<0.05

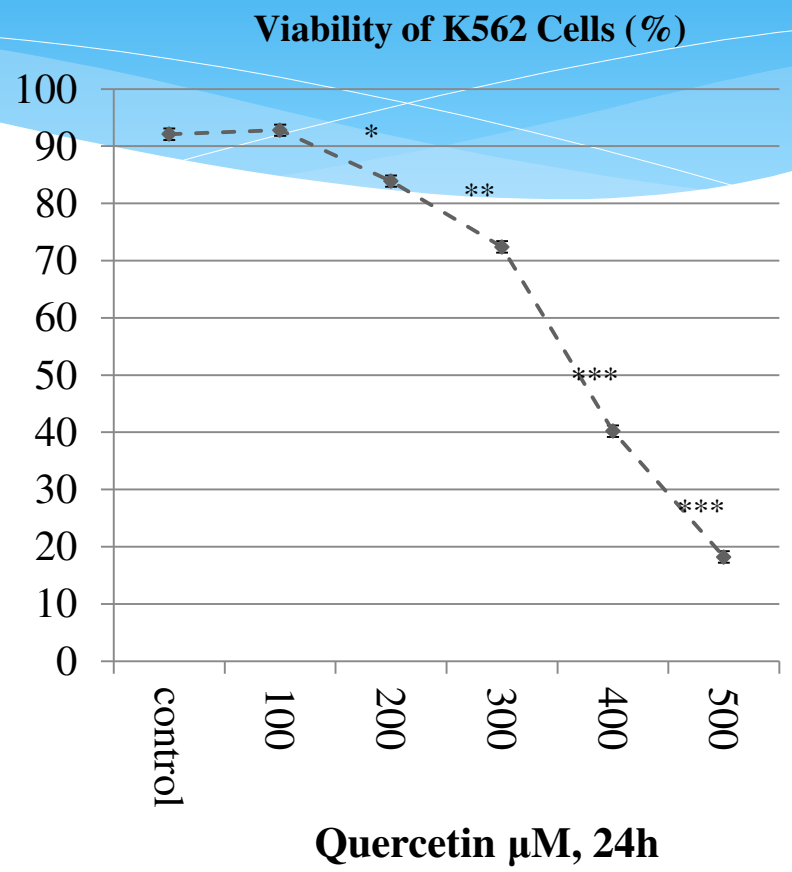


Figure 2. Effect of Quercetin Toxic Doses Over the K562 Cells Viability for 24 Hours. Non-apoptotic cells rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. *p<0.05, **p<0.01, ***p<0.001

- In third probe, hydrogen peroxide (H2O2) was applied in dose 150, 300 and 600 µM for 24h. Kind of ROS, hydrogen peroxide (H2O2) induced apoptosis of K562 cells had been shown previously and similar results was obtained in our study ($p<0.01$) (Figure 3).
- To determine preventive role of quercetin (free radical scavenger flavonoid) against to H2O2 (ROS) induced apoptosis, 100 µM of quercetin was added into cell culture medium, **20 minute before the 150, 300, 600 µM H2O2 treatment.**
- K562 cells viability were maintained by the Quercetin from the H2O2 induced apoptosis ($p<0.01$), (Figure 3).

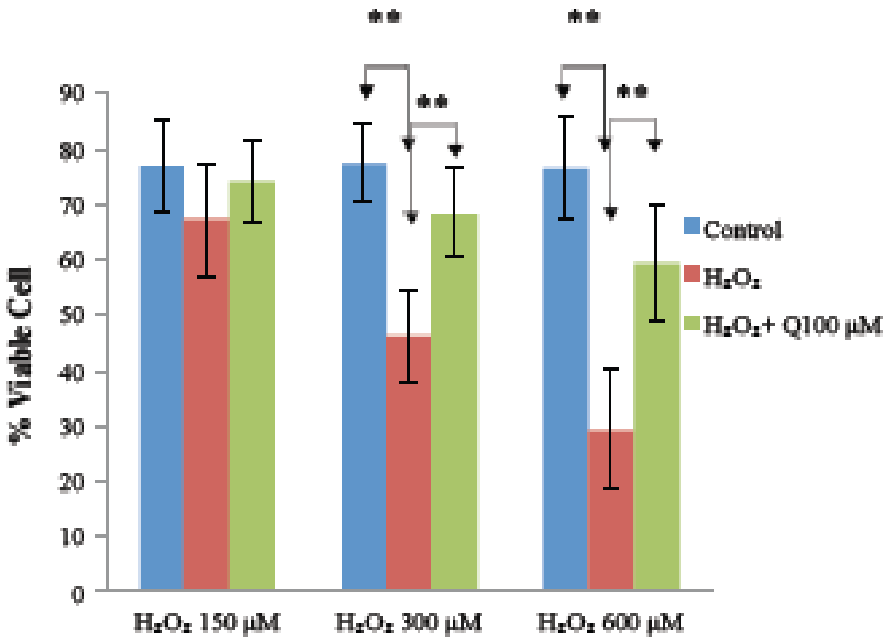
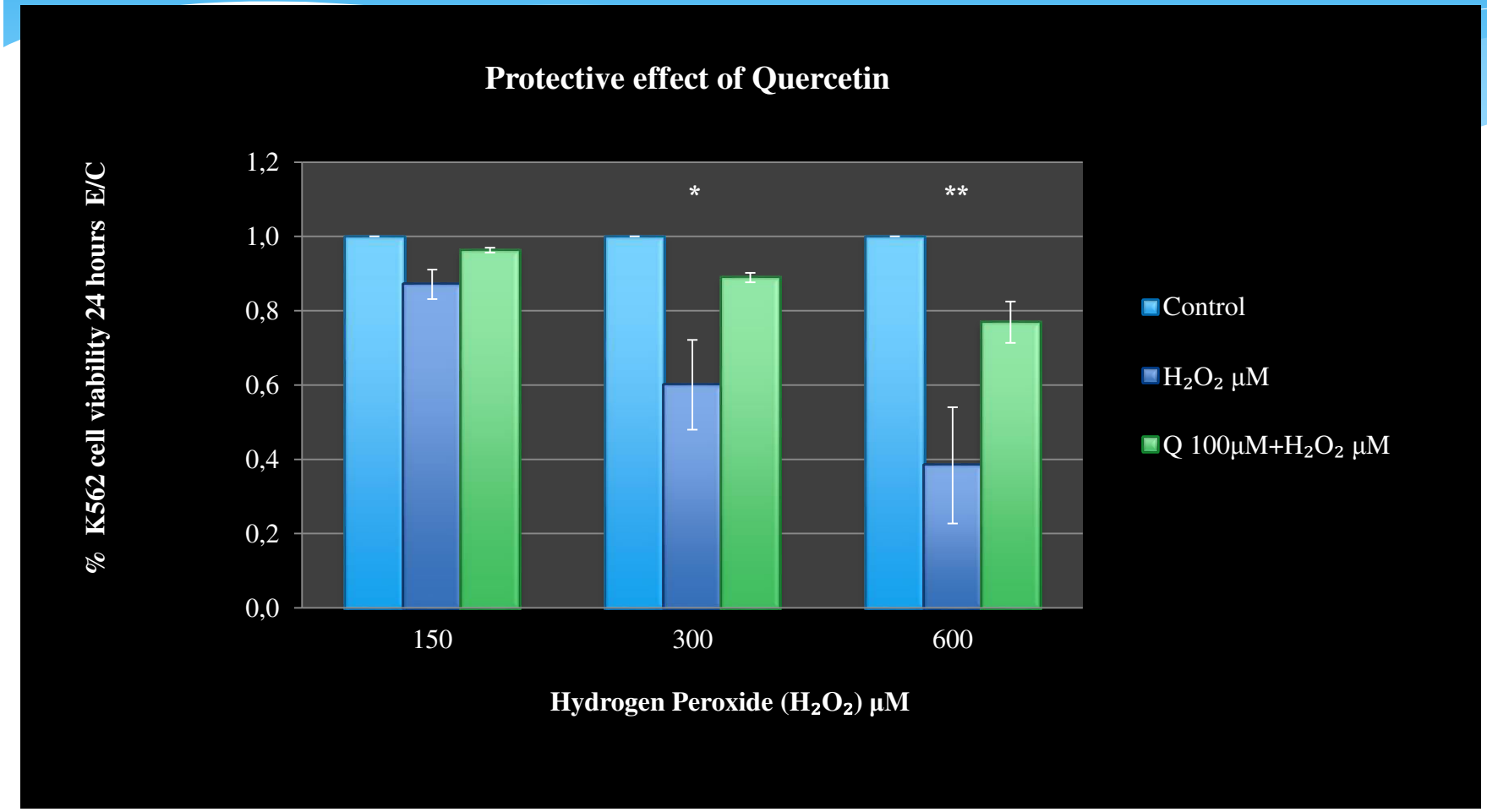


Figure 3. Protective Effect of Quercetin Over the H_2O_2 Induced Apoptosis of K562 Cell Line for 24 Hours. Non-apoptotic cell rate (% viable cells) was assessed by FITC-Annexin V/PI flow cytometer assay. **p<0.01


Protective effect of Quercetin
% K562 cell viability 24 hours E/C
1,2
1,0
0,8
0,6
0,4
0,2
0,0
*
**
150
300
600
Hydrogen Peroxide (H_2O_2) µM
Control
H_2O_2 µM
Q 100µM+H_2O_2 µM

Discussion

* In previous studies quercetin was implied as an apoptotic activator and antioxidant as well as protective agent against various types of cancer (Jung et al., 2010). It was shown that quercetin can inhibit proliferation of tumor cells and reduce the number of aberrant crypt foci in colon tumors (Van Erk et al., 2005) that it can facilitate programmed cell death in lung carcinoma (Nguyen et al., 2004) and colonorectal tumor cells (Richter et al., 1999). Other researchers had showed that quercetin acts as a free radical scavenger and protects cells from oxidizier molecules (Orsolic et al., 2007; Benkovic et al., 2009).
* As known, oxidative stress and free radicals are important activators for apoptosis (Rinaldi et al., 2009).
* On the other hand, studies conducted in last ten years showed that quercetin can act as an antiapoptotic agent as well (Ishikawa and Kitamura, 2000). In a few studies it has been shown that quercetin can partially prevent H2O2 - induced apoptosis (Chow et al., 2005) and it was suggested that protective effects of quercetin against oxidative injuries of some cells may be achieved via modulation of mitochondrial dysfunction and inhibition of caspase activity (Park et al., 2003).

* In this study, our results showed that higher dose of quercetin than 200 µM reduce cell viability (which considered as toxic doses by Cao et al. (2007) but low doses of quercetin (<200 µM) can increase cell viability and considered as therapeutic dose.

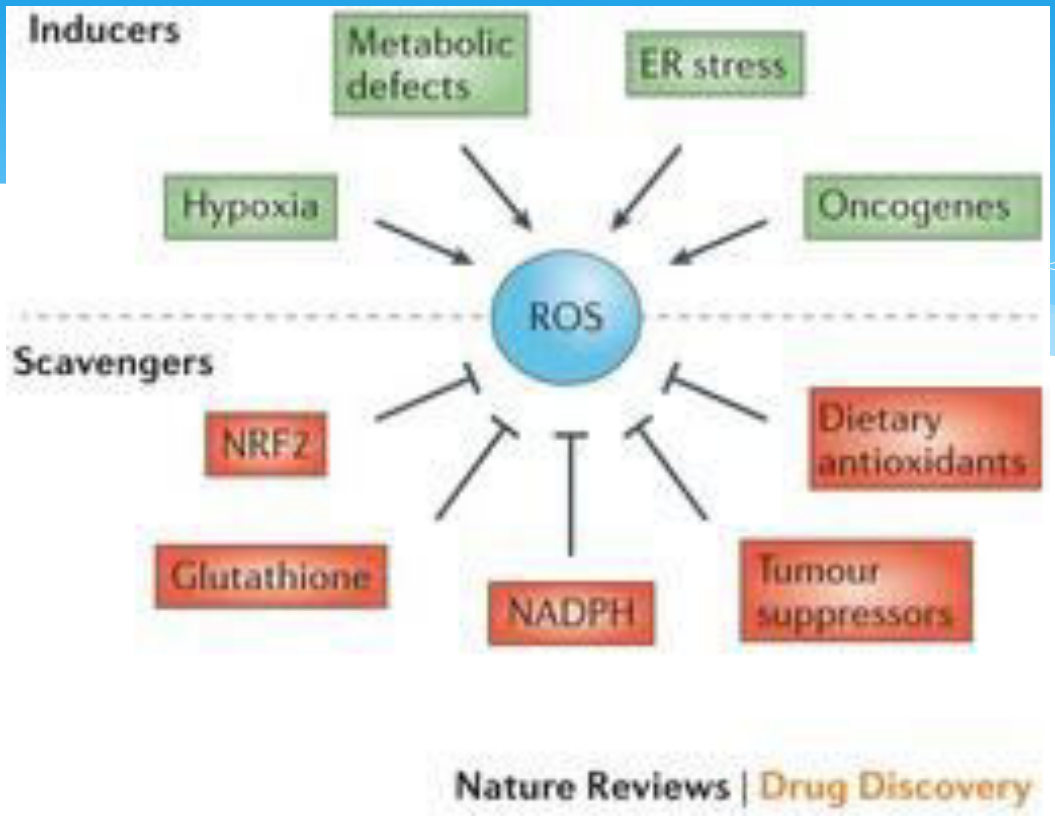
* **As it known, hydrogen peroxide is an important apoptotic mediator over mitochondrial dependent apoptotic mechanism; we also showed protective effect of quercetin over the K562 cells which induced to apoptosis with H_2O_2.**

* Free radical generation and inactivation's are under the strict control of oxidoreductive reactions. Excessive superoxide production can accelerate cell death, DNA damage and can lead to cancer, also excessive inactivation of superoxides can disturb apoptosis signals.

* Inactivation of radiation and environmental sourced superoxides by the herb sourced flavonoid free radical scavengers seems beneficial for cancer prevention (Murakami et al., 2008) but if cancer is already present, as known as free radicals are important for apoptosis signals.

* **For this reason, uncontrolled consumption of flavonoid free radical scavengers such as quercetin during chemotherapy and radiotherapy may weaken the effect of chemotherapeutics and radiotherapy rather then help it.**

* It has been theorized that cancer risk reduction may be achieved by greater consumption of phytochemical-rich fruits and vegetables (Davis et al., 2009; Rinaldi et al., 2009). However, results of our research suggest that antioxidants can interfere and attenuate success of anticancer therapy so antioxidant intakes should be strictly controlled in the cancer diagnosed patients.



The production of reactive oxygen species (ROS) can be induced by hypoxia, metabolic defects, endoplasmic reticulum (ER) stress and oncogenes. Conversely, ROS are eliminated by the activation of the transcription factor nuclear factor

Chiara Gorrini, Isaac S. Harris & Tak W. Mak. Modulation of oxidative stress as an anticancer strategy, Nature Reviews Drug Discovery 12, 931–947 (2013) doi:10.1038/nrd4002

All over that, we could say, cancer prevention and therapy are totaly different processes and aproaches should be different

Three important point may be life saver with cancer struggle

* The right diet for cancer prevention; intake of natural antioxydant flanovoids
* Early detection and Rapid Intervention of Cancer
* The right diet during cancer therapy with Sugar and antioxydant free feedings and Protein enriched products

THANKS

* Z Akan, et al,. Antioxidants may protect cancer cells from apoptosis signals and enhance cell viability. Asian Pacific Journal of Cancer Prevention 14 (8), 4611-4614



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