International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

Effect of Quercetin on Nutrient Mixture for cancer prevention

Kiran Fulzele[1], Swati Kotwal[2]

[1]*Assistant Professor, Department of Chemistry, Fergusson College, Pune University, Pune (India)*

[2]*Retired Professor, PGTD of Biochemistry, RTM Nagpur University, Nagpur (India)*

ABSTRACT

Cancer is a complex disease involving combination of genetic and epigenetic alterations.The standard treatments like chemotherapy, radiation and surgery introduces strong medicines, heat energy which target fast-growing cells, but this also includes normal events such as hair growth and digestion. Surgical removal of tumor leads to permanent recovery, but undetected malignant cells may have been already metastasized to other organs or been loose by the surgery itself.

This situation demands development of newer strategies in the treatment.The alternative therapies include antioxidant, nutrient, natural and biology based practices, interventions and products. The use of these phototherapies has gained importance in recent times because of its action in prevention of many life threatening, critical and degenerative diseases.

It has been reported that many nutrients possess anticancer activity and their combinations can enhance their efficacy. Our aim was to study whether the nutrients and phytochemicals can be used as an adjuvant or as curative therapy in the treatment of cancer. We planned to use the combination of nutrients and quercetin. Some nutrients which are essential for the formation of healthy extra cellular matrix were showing pro-proliferative effect whereas selenium, NAC and GTE were showing antiproliferative effect. Synergistic effect of these nutrients and quercetin showed 77% to 96% antiproliferative effect on cancer cell lines.

Keywords: Anticancer, Anti-proliferative, Antioxidant, cancer cell lines, phytotheraphy.

I. INTRODUCTION

Cancer is a multifactorial, multifaceted and multidimensional disease requiring a multidimensional approach for its treatment, control and prevention.Cancer being multistep process, its treatment depends upon the type of cancer, stage of cancer, and overall condition. Thus effective cancer cure should ideally target the stoppage of tumor growth, its degeneration, its disappearance completely and no recurrences.

Treatment for cancer ranges from rounds of powerful chemicals to focused burst of radiation to complete surgical removal of the tumor and surrounding tissue with a certain level of risk and pain to the patient. All the standard treatments are associated with severe toxicity with the development of drug resistance by the cancer cell, severe side effects and development of new cancer.

This situation demand for the development of new strategies in the treatment of cancer aimed at increasing the efficacy of treatments, as well as reducing drug and radiation toxicity, and developing new therapies. The

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

alternative therapy includes antioxidant, nutrients, natural and biology based practices, interventions and products. Many overlap with conventional medicine's use of dietary supplements.

In 1992 Rath and Pauling postulated that nutrients such as lysine and ascorbic acid could act as natural inhibitors of extra cellular matrix (ECM) proteolysis and, modulate tumor growth and expansion. These nutrients can exercise their anti-tumor potential through several mechanisms, among them the inhibiting of MMPs and strengthening of connective tissue surrounding cancer cells (tumor-encapsulating effect). The nutrient mixture (NM) containing lysine, proline, ascorbic acid, and green tea extract also suppressed the growth of these tumors. In the currentstudy, we investigated the anti-tumor potential of NM and quercetin on the human breast cancer cell line MDA-MB231 we added Quercetin to the nutrient mixture (NM). Quercetin significantly affects antiproliferative effect of NM. In MDA-MB 231 addition of 200ng/ml Quercetin increased % inhibition from 80 to 94%.

II. MATERIALSANDMETHODS

2.1 **chemicals**- Leibovitz's (L-15), McCoy's 5A (Hyclone) dimethyl sulphoxide (DMSO), phosphate buffer saline, EDTA, trypsin, L-Glutamine, pH (7.4 to 7.6) (Hyclone), Phenol red (0.5%) (Himedia), Trypan Blue (0.4%) (Himedia), 10% Fetal calf serum FCS (Hyclone), 0.1% Antibiotic solution (Hyclone), MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} (sigma), Matrigel invation kit, L-Lysine, L-proline and L-Arginine. Vitamin C, Selenium, N-Acetyl Cystein and Epican Forte (TABLE1).

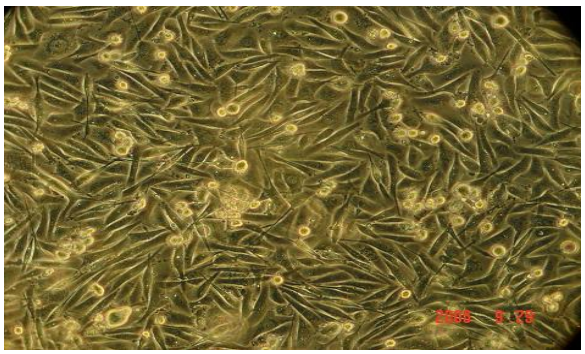
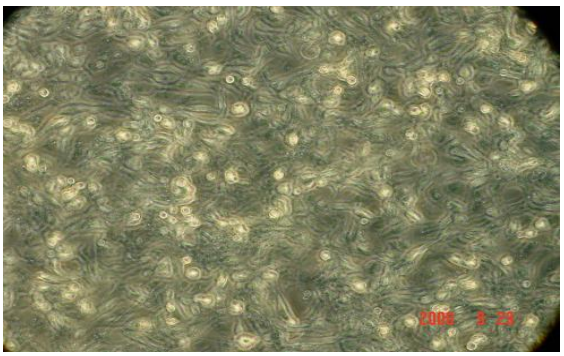
TABLE: 1 Composition of Epican Forte.

4.5g of Epican Forte will provide		
1	Ascorbic acid (mg)	710
2	L-Lysine (mg)	1000
3	L-Proline (mg)	750
4	L-Arginine (mg)	500
5	Epigallocatechin Gallate (mg)	350
6	N-Acetyl Cystein (mg)	200
7	Selenium (ug)	30
8	Copper (mg)	2
9	Manganese (mg)	1
10	Calcium (mg)	22
11	Magnesium (mg)	50

2.2 **Cell lines and culture** - MDA-MB 231, human breast carcinoma, originated from a human metastatic ductal breast carcinoma sample of a 48-year-old woman with breast carcinoma in 1976 (fig.1) and HT-29**,** Human colon adenocarcinoma originated from the primary tumor of a 44-year-old Caucasian woman with colon adenocarcinoma in 1964; described to be heterotransplantable forming well differentiated grade I tumors (fig. 2)were obtained from National Centre for Cell Science, Pune, India. The cell lines were grown on 90% Leibovitz's (L-15) medium containing L-Glutamine, pH (7.4 to 7.6) (Hyclone) + 10% Fetal calf serum FCS (Hyclone) + 0.1% Antibiotic solution (Hyclone).

Fig:1: Photograph of MDA-MB 231 cell line Fig: 2: Photograph of HT 29 cell line

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

2.3**Cytotoxicity activity** - Nutrient mixture (NM) was subject to in vitro anticancer activity against human cancer cell lines. In brief the cells were grown in tissue culture flasks in growth medium at 37ºC in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100µl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue plate. The cells were allowed to grow for 24 hours. NM was then added to the wells and cells were further allowed to grow for another 48 hours.The serial dilution of the cytotoxic drugs in growth medium was prepared. After drug addition the plates were again incubated in a humidified atmosphere at 37^0C for 96 hours withchange in treatment and medium after every 24 hours for 96 hours of exposure period to avoid the cells exposure from the degrading toxic material. At the end of the drug exposure period, the medium was removed from all the wells containing the cells, and the cells were feeded with 200ul of fresh medium and 50ul of MTT to all the wells in the column.The plates were wrapped in aluminium foil, and incubated for 4 hours in a humidified atmosphere at 37^0C. After 4 hours the medium and MTT was removed from the wells and the MTT-formazan crystals were dissolved by adding 200ul of DMSO to all the wells in the plate. 25ul of glycine buffer was also added to all of the wells containing DMSO. Finally the absorbance is recorded at 570nm immediately, since the product is unstable.

2.4 **Matrigel Invasion Chamber Assay** - Matrigel is a basement membrane matrix composed of extracellular matrix proteins from mouse sarcoma tumor. Matrigel contains not only basement components (collagens, laminin, and proteoglycans) but also matrix degrading enzymes/their inhibitors and some growth factors and other components that allow cells, i.e. neurons, to regenerate. Invasion of tumor cells into matrix has been used to characterize involvement of ECM receptors and matrix degrading enzymes, which play roles in tumor progression. The matrix can affect gene expression is some cells as well. The efficiency of the matrigel is highly dependent on temperature.

The matrigel is thawed at 4^0C overnight and then allowed it to come to room temperature.Allowed to rehydrate for 2 hours in 37^0C in incubator by adding bicarbonate. The medium was removed and chemoattractant was added to the companion well. Cell suspension was prepared in culture medium containing 50,000cells/well.

The insert chamber was then transferred to wells of companion plates with sterile forceps. Immediately 0.5ml of cell suspension was added to the chambers containing 50,000 cells/well. After 4 hours the cells were washed with basal medium and then the treatment to the cells were given and incubated for 24 hours at 37^0C in incubator.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

2.4.1 **Measurement of cell invasion** - Non-invading cells from the upper surface of the membrane were removed by scrubbing. A cotton tipped swab was inserted into the inserts and a gentle but firm pressure was applied while moving the tip over the membrane surface. The scrubbing was repeated with second swab moistened with medium.

2.4.2 **Staining of the cells** - Added 100% methanol to appropriate number of wells of companion plate.In a separate plate 1% toluidine blue in 1% borax was added. Sequentially the inserts were transferred through methanol, toluidine and 2 beakers of water for approximately 2 minutes in each solution. Then the inserts were allowed to dry and counting of invading cells were done at approximately 40-200X magnification

III. STATISTICAL ANALYSIS

All of the experiments were performed in triplicate and wererepeated at least three times. Data were expressed as mean values± SE and were analyzed by ANOVAfollowed by the Tukey's multiple comparison; the levelof significance was $P< 0.05$.

IV. RESULTS AND DISCUSSION

Literature was surveyed to determine the achievable plasma concentration of the nutrients and then concentrations of each nutrient were selected (TABLE no 2).

Quercetin being inhibitor of various phase I and phase II enzyme as well as phase III transporters, has been reported to act as a bioenhancer. We have shown in other study in our laboratory [1] that quercetin increases systemic availabilityof EGCG in rats and human. We wanted to test whether quercetin addition to treatment mixture causes significant increase in inhibition of cell proliferation. The concentration tried was 100ng; 200ng and 500ng. TABLE 3 shows theeffect of different levels of quercetin on MDA-MB 231 and fig 3 shows the effect of different levels of quercetin with APL + L-Arg onMDA-MB 231

TABLE 2 Different Concentration of Nutrients

Nutrients	**µM**
Ascorbic Acid	100
L-Lysine	400
L-Proline	140
L-Arginine	400
N-Acetyl Cystein (NAC)	50
EF	5
Selenium	2

TABLE 3 Effect of combining different level of Quercetin with APL + L-arginine on cell proliferation of MDA-MB 231.

Sr. no	Supplements*	% Effect (Mean±SE)	% inhibition against control	(S) or (NS)
1	control	100 ± 0.00	0	-
2	100ng Q	58.0 ± 3.61	***-42.0***	-
3	200ng Q	64.3 ± 2.03	***-37.5***	NS
4	500ng Q	53.3 ± 0.33	***-46.7***	NS
5	APL + L-Arg + 100ng Q	47.7 ± 3.67	***-52.3***	NS
6	APL + L-Arg + 200ng Q	60.7 ± 4.37	***-39.3***	NS
7	APL + L-Arg + 500ng Q	41.0 ± 2.00	***-59.0***	NS

S- significant, NS- non significant

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

The above experiment done shows the effect of graded concentration of quercetin on MDA-MB 231. There was no significant difference by higher level of quercetin when compared with lower concentration. 100ng Q alone gives 42% cell inhibition, 200ng Q gives 37.5% cell inhibition and 500ng Q the highest level tried in this experiment gives 46.7% cell inhibition which was not significant against 100ng Q. After combining it with APL + L-Arg the cell inhibition with 100ng Q and 500ng Q was increased i.e. 52.3% and 59.0% respectively but was decreased with 200ng Q and was 39.3%. Since no significance was obtained between this graded concentrations of quercetin hence to know a concentration which is beneficial in controlling the cell growth we tried each concentration with different nutrients.

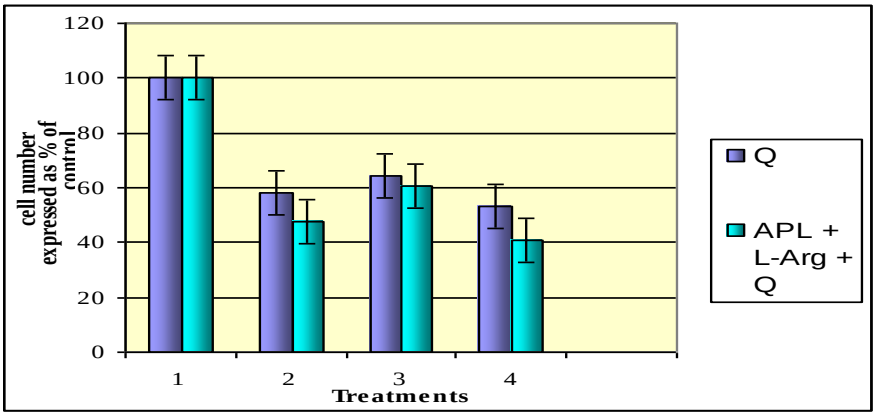


Fig 3 Effect of different level of quercetin with APL + L-Arg on MDA-MB 231.

Next experiment was planned with 100ng Qwith different nutrients. Table 4 shows the next combination tried.

TABLE 4Effect of combining 100ng Quercetin, EF, APL, L-Arginine, Selenium and NAC on cell proliferation of MDA-MB 231.

Sr. no	Supplements*	% Effect (Mean±SE)	% inhibition against control	Significant against treatment No:2
1	Control	100 ± 0.00	0	-
2	100ng Q	58.0 ± 3.61	***-42.0***	-
3	5ug EF	82.7 ± 1.86	***-17.3***	NS
4	100ng Q + 5ug EF	61.0 ± 2.65	***-39.0***	NS
5	100ng Q + 5ug EF + APL + L-Arg	52.7 ± 2.33	***-47.3***	NS
6	100ng Q + 5ug EF + APL + L-Arg + 50uM NAC	39.0 ± 2.08	***-61.0***	***S***
7	100ng Q + 5ug EF + APL + L-Arg + 50uM NAC + 2uM Sel	22.3 ± 3.18	***-77.7***	***S***

100ng Q as tried in the previous experiment gives 42% cell inhibition and 5ug EF gives 17.3% cell inhibition. Quercetin alone was significant against EF. 100ng Q when supplemented with 5ug EF gives 39% cell inhibition and was significant against 5ug EF. When APL + L-Arg were supplemented to the above treatment of 100ng Q + 5ug EF the cell inhibition was increased to 47.3%. This treatment was significant against treatment no; 3 and 4. APL + L-Arg + 5ug EF showed 9% cell inhibition which was increased to 47.3% after addition of quercetin. 61% cell inhibition was obtained when 50uM NAC was added to the treatment no; 5 containing 100ng Q + 5ug EF + APL + L-Arg. Thiscell inhibition was doubled after addition of quercetin. 5ug EF + APL + L-Arg + 50uM NAC showed inhibition of 30%. This treatment was significant against treatment no; 2 and 3. Highest cell

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

inhibition of 77.7% was obtained after addition of 2uM selenium in 100ng Q + 5ug EF + APL + L-Arg + 50uM NAC. This treatment was significant against treatment no; 2 (100ng Q) and 3(5ug EF). Treatment no; 6 (100ng Q + 5ug EF + APL + L-Arg + 50uM NAC) and treatment no; 7 (100ng Q + 5ug EF + APL + L-Arg + 50uM NAC + 2uM Sel) was significant against treatment no; 2 (100ng Q) and treatment no; 3 (5ug EF).

This experiment proves that addition of quercetin enhances cell inhibition and support the above said statement that it acts as a bioenhancer [1].

Next experiment was tried with 200ng quercetin. TABLE 5 shows the combination tried with 200ng quercetin.

TABLE 5 Effect of combining 200ng Quercetin, EF, APL, L-Arginine, Selenium and NAC on cell proliferation of MDA-MB 231.

Sr. no	Supplements*	% Effect (Mean±SE)	% inhibition against control	Significant against treatment No:2
1	Control	100 ± 0.00	0	-
2	200ng Q	64.3 ± 2.03	*-35.7*	-
3	5ug EF	82.7 ± 1.86	*-17.3*	NS
4	200ng Q + 5ug EF	50.0 ± 1.15	*-50.3*	*S*
5	200ng Q + 5ug EF + APL + L-Arg	43.0 ± 0.58	*-57.0*	*S*
6	200ng Q + 5ug EF + APL + L-Arg + 50uM NAC	47.0 ± 1.15	*-53.0*	*S*
7	200ng Q + 5ug EF + APL + L-Arg + 50uM NAC + 2uM Sel	28.0. ± 3.06	*-72.0*	*S*

200ng quercetin gives 35.7% cell inhibition and 5ug EF gives 17.3% cell inhibition same as tried in the previous experiment. Treatment no; 2 (200ng Q) was significant against treatment no; 3 (5ug EF). Supplementing 200ng quercetin with 5ug EF increased the cell inhibition to 50.3%. But when treatment no 7 was compared to treatment no 7 in table 4, increasing 100ng Q to 200ng Q did not cause significant increase in inhibition. This treatment was significant against both treatment no; 2 (200ng Q) and 3 (5ug EF). When APL + L-Arg was supplemented to treatment no; 4 (200ng Q + 5ug EF) cell inhibition was again increased and was 57%. This was also significant against treatment no; 2 (200ng Q) and treatment no; 3 (5ug EF). After addition of 50uM NAC the cell inhibition instead of increasing was decreased by 4% and was 53.0%. This treatment too was significant against treatment no; 2 (200ng Q) and 3 (5ug EF). 2uM selenium showed the highest cell inhibition of 72% when added to treatment no; 6 (200ng Q + 5ug EF + APL + L-Arg + 50uM NAC). This also showed significance against treatment no; 2 and 3.

Next treatment was tried with 500ng quercetin. TABLE 6 show the combination tried.

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

TABLE 6 Effect of combining 500ng Quercetin, EF, APL, L-Arginine, Selenium and NAC on cell proliferation of MDA-MB 231.

Sr. no	Supplements*	% Effect (Mean±SE)	% inhibition against control	Significant against treatment No:2
1	Control	100 ± 0.00	0	-
2	500ng Q	53.3 ± 0.33	-46.7	-
3	5ug EF	82.7 ± 1.86	-17.3	NS
4	500ng Q + 5ug EF	40.3 ± 2.03	-59.7	S
5	500ng Q + 5ug EF + APL + L-Arg	33.7 ± 0.88	-66.3	S
6	500ng Q + 5ug EF + APL + L-Arg + 50uM NAC	36.3 ± 0.67	-63.7	S
7	500ng Q + 5ug EF + APL + L-Arg + 50uM NAC + 2uM Sel	3.7 ± 2.33	-96.3	S

This experiment shows the effect of 500ng quercetin with different nutrients. It showed similar result as that obtained by 200ng quercetin. 500ng quercetin alone gives cell inhibition of 46.7% and 5ug EF gives 17.3% of inhibition. 500ng quercetin was significant against 5ug EF. On combination (500ng Q + 5ug EF) the cell inhibition was increased to 59.7%. This treatment was significant against both 500ng quercetin and 5ug EF. Addition of APL + L-Arg further increased the cell inhibition to 66.3% with significance against 500ng quercetin and 5ug EF. The cell inhibition was reduced to 2.3% on addition of 50uM NAC. 63.7% cell inhibition was obtained when 50uM NAC was supplemented with 500ng Q + 5ug EF + APL + L-Arg mixture. Highest cell inhibition of 96.3% was obtained on addition of 2uM selenium in treatment no; 6 (500ng Q + 5ug EF + APL +L-Arg + 50uM NAC). This was significant against treatment no 7 in table no 4 and 5. Availability of polyphenols is low it is therefore helpful to formulate nutrients mixtures containing less quantity of polyphenols. The next was to find out the concentration which gave most inhibitory action. TABLE 7 and fig 4 shows the inhibitory effect obtained by 100ng, 200ng and 500ng quercetin.

TABLE 7 Inhibitory activities obtained with 100ng Q, 200ng Q and 500ng Q with APL + L-Arg along other agents.

		Inhibition against quercetin		
Sr. no	Supplements*	100ng Q	200ng Q	500ng Q
1	Q	-	-	-
2	5ug EF	NS	NS	NS
3	Q + 5ug EF	NS	*S*	*S*
4	Q + 5ug EF + APL +L-Arg	NS	*S*	*S*
5	Q + 5ug EF + APL + L-Arg + 50uM NAC	*S*	*S*	*S*
6	Q+ 5ug EF + APL + L-Arg +50uM NAC + 2uM Sel	*S*	*S*	*S*

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 09 , September 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

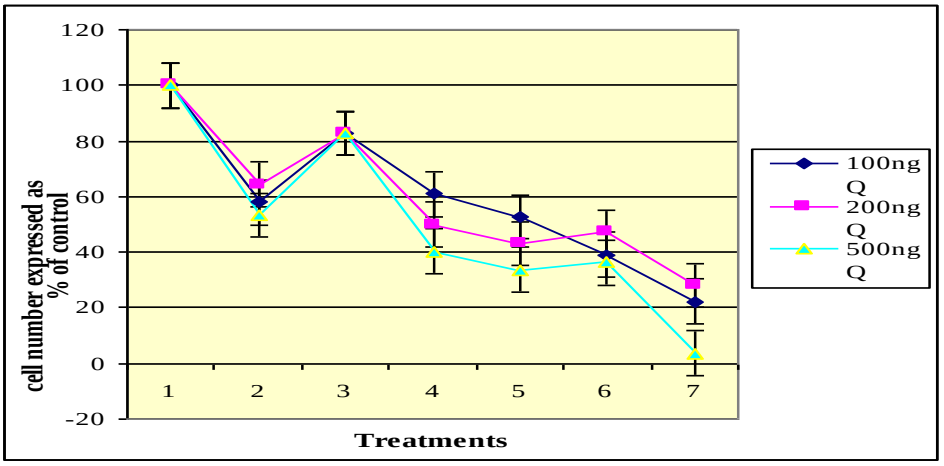


Fig 4 Shows the inhibitory effect obtained by different levels of Quercetin.

This table shows the inhibitory effect obtained between 100ng, 200ng and 500ng quercetin. 100ng quercetin is giving only 2 treatments significant against rest of the treatment where as 200ng and 500ng quercetin is showing 4 significant results when compared with rest of the treatment.

The result obtained by quercetin was nearly same for the three different concentrations. 200ng and 500ng quercetin gave the same results. It exerts antiproliferative action against many cancer cell lines [2]. Quercetin is reported to be an inhibitor of UDP- glucuronyl trsnsferase (UGT). It is also reported that quercetin could competitively inhibit the members of MDR family, P-gp, MRP1 [3] and BCRP [4],[5],[6], [3]. Quercetin has been found to inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 including CYP3A4 the enzyme involved in the metabolism of most of the therapeutic drugs [7], [8], [9], [10]. The co-administration of quercetin significantly increases the Ka, AUC and Cmax of 4-hydroxytamoxifen [11] The co-administration of quercetin enhanced the oral bioavailability of tamoxifen decreasing the efflux by MDR family transporters intestine, liver and kidney as well as reducing the first-pass metabolism of tamoxifen and also that of digoxin [11], [12].

We also tried the matrigel invasion study here. The matrigel invasion studies were conducted with colon cancer cells HT-29. Here we studied the effect of quercetin and EF on HT-29 migration. The concentration tried for quercetin and EF is given in table 3.40.

Table 8 The concentration of quercetin and EF on HT-29 cell migration.

Sr. No	Supplements	% Effect (Mean+SE)	% of cell migrated	Significant against control
1	Control	100 ± 0.000	100%	-
2	10ug/ml EF	94.2 ± 0.35	94.5%	*S*
3	5ug/ml EF + 500ng/ml Q	75.1 ± 0.00	75%	*S*
5	500ng/ml Q	75.1 ± 2.00	75%	*S*

Contrary to expectation the addition of 10ug of EF to the media did not have any effect on the cell migration of HT-29. Addition of 500ng Q decreased the matrigel invasion by the cells to 75%. The inhibition of invasion by the cancer cells was still seen when 500ng was added to the media containing 5ug EF. It is noteworthy here that when EF was used alone it needed at least 10ug/ml EF in the media for causing significant inhibition of invasion. EF is a combination of ascorbic acid, lysine, proline, green tea extract, arginine, NAC, selenium,

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

copper and manganese which work synergistically at concentration approximately those found in the blood of healthy individual inhibiting the migration of cancer cells through matrigel membrane by 50%, 10% and 30% in breast cancer cells (MDA-MB 231), melanoma cells (A 2058) and colon cancer cells (HCT116) respectively [13]. Netke et al (2003) found that when 20ug/ml of EGCG reduced the number of MDA-MB 231 cells migrate through matrigel to about 70%,where as 800ng/ml EGCH in EF reduced the number of migrating cells to 53% [14]. EF was found to be effective in a variety of cancer cell types, including solid tumors and the cells involved in (leukemia and HTLV-1 virus-derived leukemia ATL) [15], [16], [17], [18], [19], [20], [21], [22], and Harakeh et al., 2004a, b). They also investigated the requirement of EF for complete inhibition (100%) of Matrigel invasion for several cell lines.

At the end of this study, it is clear that 100uM ascorbic acid, 400uM L-lysine, 140uM L-proline, 400uM L-arginine, 1.5uM and 2.0uM selenium, 50uM NAC, 10ug EF plus 100ng quercetin is the best nutrient mixture to achieve maximum inhibition of MDA-MB 231. More pronounced inhibition was obtained in some treatment but those treatments were rejected on grounds that they were high concentration not achievable in plasma. This combination will now be used with other cell lines to assess its universal usability.

REFERENCES

[1] Kale A, Gawande S, Kotwal S, Netke S, Roomi W, Ivanov V, Niedzwiecki A, Rath M, Studies on the Bioenhancing effects of onions and other nutrients on the absorbtion of Epigallocatechin gallate from green tea extract in human volunteers. 2nd International conference on Tumor Progression & Therapeutic Resistance Proceedings, 2007, page **89.**

[2] Talcott SU, Talcott ST, Percival SS. Low concentrations of Quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells,J Nutr , 2003; **133**: 2669-2674.

[3] Van Zanden JJ, Wortelboer HM, Bijlsma S, Punt A, Usta M, Bladeren PJ, Rietjens IM, Cnubben NH. Quantitative structure activity relationship studies on the flavonoid mediated inhibition of multidrug resistance proteins 1 and 2. Biochem. Pharmacol, 2005, **69:** 699-708.

[4] Cooray HC, Janvilisri T, Van Veen HW, Hladky SB, Barrand MA. Interaction of the breast cancer resistance protein with plant polyphenols. Biochem. Biophys. Res. Commun. 2004, **317**: 269-275.

[5] Scambia G, Reneletti FO, Panici PB, De Vincenzo R, Bonanno G, Ferrandina G, Piantelli M, Bussa S, Rumi C, Cianfriglia M. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. Cancer Chemotherapy Pharmacol; 1994, 34: 459-464.

[6] Sarkar MA. Quercetin not only inhibits P-glycoprotein efflux activity but also inhibits CYP3A isozymes. Cancer Chemother Pharmacol; 1995, 36: 448-450.

[7] Obach et al. Inhibition of Human Cytochrome P450 Enzymes by Constituents of St. John`s Wort, an Herbal Preparation Used in the Treatment of Depression. Pharmacologyand Experimental Therapeutics; 2000, **294(1)**: 88-95.

[8] Hsiu SL, Hou YC, Wang YH and others. Quercetin significantly decreased cyclosporine oral bioavailability in pigs and rats. Life Sci; 2002,**72**: 227-35.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

[9] Reid et al. Metabolic activation of dacarbazine by human cytochromes P450: the role of CYP1A1, CYP1A2 and CYP2E1. Clin Cancer Res, 19995: 2192-2197.

[10] Bacon et al. Sulforaphane and Quercetin modulate PhIP±DNA adduct formation in human HepG2 cells and hepatocytes. Carcinogenesis; 2003, **24**: 1903-1911.

[11] Shin CS, Choi JS and Li X. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with Quercetin in rats. International Journal ofPharmaceuticals, 2006 **313**: 144-149.

[12] Wang YH, Chao PD, Hsiu SL, Wen KC, Hou YC. Lethal Quercetin-digoxin interaction in pigs. Life Sci; 2004, **74**: 1191-1197.

[13] Netke SP, Roomi MW, Ivanov V, Niedzwiecki A, Rath M.A, Specific combination of ascorbic acid, lysine, proline and epigallocatechin gallate inhibits proliferation and extracellular matrix invasion of various human cancer cell lines. Research Communication in Pharmacology and Toxicology; Emerging Drugs Vol. II, 2003: 37-50.

[14] Roomi MW, Netke SP, Ivanov V, Rath M, Niedzwiecki A. Epican Forte. A Specific formulation of nutrients containing lysine, proline, ascorbic acid and epigallocatechin gallate inhibits matrix metalloproteinase activity and invasion potential of human cancer cell lines. Paper presented at European Organization for Research and Treatment of cancer, American Association of Cancer Research and National Cancer Institute Symposium on molecular Targets and cancer Therapeutics, Frankfurt, Germany, 2002, Nov 19-22.

[15] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M, Antiangiogenic activity of a novel formulation of lysine, proline, ascorbic acid, green tea extract and other in preparation. 2004.

[16] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. In vitro and in vivo antitumorogenic activity of mixture of lysine, proline, ascorbic acid and green tea extract on human breast cancer cell line MDA-MB 231 and MCF 7. Med. Oncol; 2005a, **22**: 129-38.

[17] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Antitumor effect of a combination of lysine, proline, arginine, ascorbic acid and green tea extract on pancreatic cancer cell line MIA PaCa-2. Int. J. Gastrointest. Cancer; **35**: 2005b, 97-102,.

[18] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. In vivo antitumor effect of ascorbic acid, lysine, proline, and green tea extract on human prostrate cancer PC-3 xenograft in nude mice: evaluation of tumor growth and immunohistochemistry. In vivo; **19**, 2005c, 179-83,.

[19] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Antitumor effect of nutrient synergy on human osteosarcoma cells U-20S, MNNG-HOS and Ewing`s sarcoma SK-ES.1. Oncol. Rep, 2005d,**13**: 253-57.

[20] Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. In vivo antitumor effect of ascorbic acid, lysine, proline, and green tea extract on human colon cancer cell HCT 116 xenograft in nude mice: evaluation of tumor growth and immunohistochemistry. Oncol Rep; 2005e,**13**: 421-425.

[21] Roomi MW, Netke SP, Ivanov V, Niedzwiecki A, Rath M. Epican Forte-A specific formulation of nutrients containing lysine, proline, ascorbic acid and epigallocatechin gallate inhibits matrix metalloproteinases activity and invasive potential of human cancer cell lines. European Cancer Journal 2002; **38(7):**2005f, Abs 280,.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

[22] Roomi MW, Roomi NW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Modulation of N-methyl-N-nitrosourea induced mammary tumors in Sprague-Dawley rats by combination of lysine, proline, arginine, ascorbic acid and green tea extract. BritishCancer Research; **7**: 2005g, R 291-R 29.

[23] Harakeh SM, Diab-Assaf M, Niedzwiecki A, Khalife J, Abu-el-Ardat K, Roomi MW, Rath M. Anti-proliferative effects of antioxidants using HTLV-1 positive and negative malignant T-cells. 104th General Meeting of the American Society for Microbilogy. New Orleans, Louisiana, May 2004 a, p-143, Abstract T-021,.

[24] Harakeh SM, Diab-Assaf M, Niedzwiecki A, Khalife J, Abu-el-Ardat K, Roomi MW, Rath M. Induction of apoptosis by Epican Forte in HTLV-1 positive and negative malignant T-cells World Conference on Dosing of Anti-infectives: Dosing the Magic Bullets. Nurnberg, Germany (oral presentation), September 9-11, 2004 b.