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"TO EVALUATE ANTICANCER ACTIVITY OF SOME AMINO ACIDS, VITAMINS, MINERALS AND FOOD CONSTITUENTS ON CANCER CELL LINES"

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Abstract: This study has been undertaken to investigate the effect of graded concentration of some amino acids, vitamins, minerals and some food constituents on 3 DIFFERENT cancer cell lines. Some nutrients like ascorbic acid, L-proline, L-lysine and L-arginine which are essential for the formation of healthy extra cellular matrix (ECM) were showing pro-proliferative effect when tried individually but were retained in the treatment mixture for their other activities that are necessary for stopping the spread of cancer. They were tried in combination with selenium, NAC and GTE which were individually showing antiproliferative. The effect of graded concentration of nutrients individually on MDA-MB 231 (breast cancer), HT-1080 (fibrosarcoma cancer) and HT-29 (colon cancer) was studied. Ascorbic acid; L-proline, L-lysine and L-arginine at the levels used in the study showed pro-proliferative effect while selenium and NAC were strongly antiproliferative

Index Terms **– Anticancer, ECM, Pro-proliferative, MDA-MB 231, HT-1080, HT-29, Ascorbic acid; L-proline, L-lysine, L-arginine, selenium, NAC.**

INTRODUCTION

Cancer is a complex disease involving combination of genetic and epigenetic alterations. It is a multifactorial, multifaceted and multi mechanism disease and hence requires a multidimensional approach for its treatment, control and prevention. Cancer involves fundamental biological processes concerning disorganized cell replication, cell death (apoptosis) and disorganization of organ structure. There are various pathways and mechanisms that lead to aberrant proliferation and apoptosis and these are the target for therapeutic intervention.

The challenge for medical research is to identify the signal or the lack of it that turns normal cell into tumor cell, control its unregulated proliferation and invasion along with suppressed cell death, inhibit proliferation of new blood capillaries (angiogenesis) to tumor site and to develop therapies that interfere or inhibit these processes. Thus, the effective therapy should ideally target stoppage of tumor growth, trigger tumor degeneration, and should not allow recurrence of cancer.

Vitamins have antioxidant activity that protects the body from oxidative damage, which is considered to be the major factor in cancer development. Ascorbic acid, abundant in green leafy vegetables, is required in the synthesis of collagen. Cancer patients are reported to show vitamin C deficiency (Anthony et al.1982, Nunez et al. 1995, Kurbacher et al. 1996, Gackowski et al, 2002) and Supplementation of vitamin C to these patients helps in strengthening the connective tissue, extra cellular matrix (ECM).

Minerals are also reported to have anti-oxidant activities. Selenium works as an anti-oxidant when combined with vitamins, by scavenging damaging particles in the body known as free radicals. It neutralizes free radicals and help in preventing the damages caused by them. Several animal and human studies show that selenium has an ability to prevent cancer cell invasion of ECM (metastasis) and affect the processes of angiogenesis and apoptosis (Fleming.et.al 2001). Use of selenium supplements in conjugation with other antioxidants including vitamin C, vitamin E, beta-carotene and coenzyme Q10 along with essential fatty acids reduces cancer spread and bring down death rate in women with breast cancer.

Some amino acids synergistically show anticancer activities, for example L-Lysine helps by inhibiting metastasis by blocking the anchor site of collagenase enzyme. L-lysine given in higher does is reported to be effective against cancer (Rath et al 1992). L-Proline is normally synthesized in the body, but the hydroxyproline content of tumor tissue is low (Chubinskaia et al 1989). Hydroxyproline excreted in the urine of cancer patients is high, this is due to the breakdown of ECM in cancer tissue and thus reduction in collagen fiber formation. L-Arginine, an essential amino acid, is required to maintain normal metabolism and proliferation of cells in culture (Hanss et al 1964). L-Arginine is precursor of nitric oxide (NO), and any deficiency of L-arginine

can limit the production of NO (Cooke et al 1997). NO acts as an inducer of apoptosis in breast cancer cells. Thus, amino acids, L-Proline and L-Lysine are required for the formation of collagen chains, ascorbic acid is essential for the hydroxylation reaction. Therefore, sufficient amount of L-proline, L-lysine and L-ascorbic acid is required for the formation of healthy and strong ECM Polyphenols, such as EGCG in green tea extract, quercetin and naringenine are very good antioxidants and have been reported to have anticancer activities.
Green tea contains higher levels of antioxidants than other tea because it is less processed and has tremendous potential in the treatment of cancer due to the powerful antioxidant

NEED OF THE STUDY.
There are various standard treatments for cancer, but they are associated with several side effects like severe toxicity, the development of drug resistance by the cancer cell, severe side effects and development of new cancer. This has necessitated development of new strategies in the treatment of cancer aimed at increasing the efficacy of treatment, as well as reducing drug and radiation toxicity and resistance. It has been reported that many nutrients possess anticancer activity and their combinations can enhance their efficacy. The new therapies include use of antioxidants, nutrients, natural phytochemicals of these phytotherapy has gain importance in recent times because of its action in prevention of many life threatening, critical and degenerative diseases (Willner Chemist 2002).
Nutrients can work synergistically was studied in Matthias Rath at San Jose, CA, USA (Netke et.al 2003). Epidemiological studies have shown that vitamins and mineral intake is closely correlated with reducing the risk of cancer (Rath et.al 1992)
Vitamins and amino acids are essential to strengthen the ECM in reducing the risk of metastasis (Rath et al 1992).

3.1 Population and Sample
Breast cancer cell line, MDA-MB-231, colon cancer cell line, HT-29, and Fibrosarcoma cell line HT-1080 were used in the study. These cell lines were purchased from National Center for Cell Science (NCCS), Pune.
All vitamins, minerals and amino acids were of highest quality and purchased from local supplier. GTE, Quercetin and naringine were purchased from Sigma. Cell culture media and other chemicals were purchased from Gibco, Hyclone and Himedia
Phenol red (0.5%) and Trypan Blue (0.4%) were purchased from Himedia. MTT {3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide} was obtained from Sigma, USA

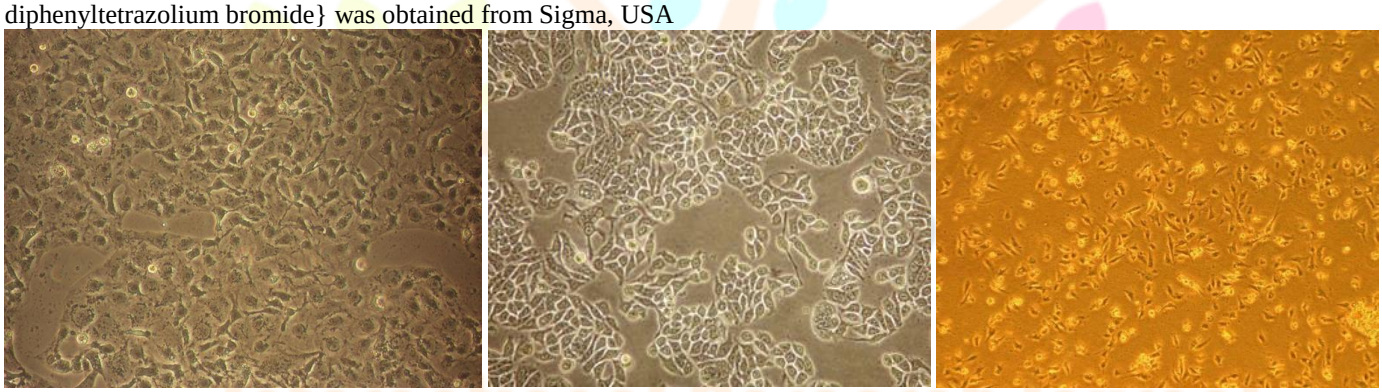

Fig 1 MDA-MB-231 HT-29 HT-1080

3.2 Data and Sources of Data
For this study secondary data has been collected from different articles and research paper. The references are given at the end of the paper.

3.3 Theoretical framework
The concentrations selected for each nutrient was finalized by literature survey and given in the table below:

Table 1 Concentration of each nutrients in uM

Nutrient	Concentration in uM				
Ascorbic acid	50	75	100	125	150
L-Lysine	200	300	400	500	600
L-Proline	60	100	140	180	220
L-Arginine	200	300	400	500	600
N-acetyl cysteine	50	75	100	125	150
Glutathione	50	75	100	125	-
Selenium	3	4	5	6	7

Daily oral administration of 1000mg of ascorbic acid has been reported to result in steady state plasma level of 80uM (Levine et al, 1996). Experiment was therefore planned around this value.
The different concentrations of ascorbic acid, L-lysine, L-proline, L-arginine, Nacetyl cystine and Glutathione tried are given in table.

RESEARCH METHODOLOGY

Cell proliferation by MTT based cytotoxicity assay:

Cells in the exponential phase of growth were exposed to various treatments. After incubation for predetermined time period, used medium was replaced with fresh medium and the cells were treated with MTT and glycine buffer and incubated for 3 hours at 37°C. The amount of MTT-formazan produced directly co-relates with the viable cells and is determined spectrophotometrically.

3.4 Statistical Analysis:

All the experiments were performed in triplicate and were repeated at least three times. Data were expressed as mean values ± SE and were statistically analyzed by one way ANOVA using "Analyse-it + general 1.63" software. P-values less than 0.05 were considered as indicative of significance.

IV. RESULTS AND DISCUSSION

4.1 Effect of graded concentration of ascorbic acid on MDA-MB 231, HT-1080 and HT-29

Table 2 Effect of ascorbic acid on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of ascorbic acid in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	50	+13.7	***-16***	***-28***
3	75	+17.7	-1.3	+8
4	100	+30.0	-9	+11
5	125	+38.3	***-24***	***-19***
6	150	+33.7	***-11***	***-15***

(# Bold and italics indicates that the values are significant when compared with control)

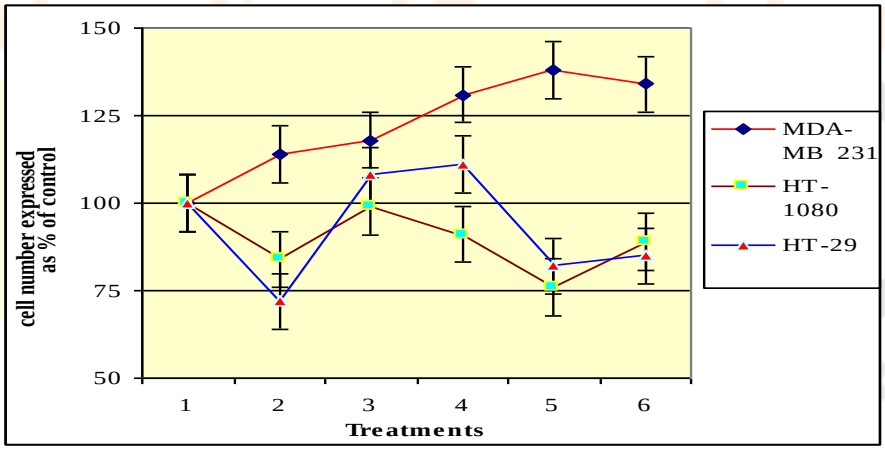

Figure 2. Effect of graded concentration of ascorbic acid on MDA-MB 231, HT-1080 and HT-29.

The response of all the three cell lines was not the same for the graded concentration of ascorbic acid as shown in table 2. It showed a pro-proliferative effect in MDA-MB 231 at all levels. There was a direct relationship between concentration and cell proliferation. As the concentration of ascorbic acid was increased the cell proliferation also increased. When compared with control group, MDA-MB 231 at 50uM (the lowest concentration tried), showed (+) 13.7% cell proliferation, at 75uM (+) 17.7% cell proliferation, for 100uM (+) 30% cell proliferation, at 125uM highest cell proliferation of (+) 38.3% was obtained. At 150uM of ascorbic acid which was highest concentration taken, the cell proliferation obtained was (+) 33.7%. The experiment shows that all the levels of ascorbic acid used caused significant increase in cell proliferation up to the level of 100uM. The increase in cell proliferation obtained at 125uM and 150uM was not significantly different from that obtained at 100uM of ascorbic acid.

The effect of ascorbic acid on HT-1080 and HT-29 showed bi-phasic effect. In case of HT-1080 at 50uM, 125uM and 150uM significantly antiproliferative effect was obtained. For 50uM (-) 16% cell inhibition, at 125uM (-) 24% and at 150uM (-) 11% cell inhibition was shown. These results were significant when compared with control groups. There was no correlation with concentration of ascorbic acid and cell inhibition. At lowest concentration it showed 16% cell inhibition and at highest concentration it showed only 11% cell inhibition whereas at 125uM concentration it showed the highest cell inhibition of 24%.

HT=-29 showed positive proliferation at 75uM and 100uM. At 50um, the lowest concentration highest cell inhibition of (-) 28% was obtained whereas (-) 19% and (-) 15% cell inhibition was obtained at 125uM and 150uM concentration respectively and was significant. The experiment thus shows that different cancer cell behave differently to graded concentration of ascorbic acid. Breast cancer cell lines shows cell proliferation at all concentration and there is direct relationship between concentration and cell proliferation. Colon cancer cell lines and fibrosarcoma cancer cell lines shows a bi-phasic effect to ascorbic acid.

Ascorbic acid has been one of the most researched nutrients and has been shown to have beneficial effect on cancer patients, (Cameron et. al. 1974). Daily oral administration of 1000mg of ascorbic acid has been reported to result in steady state level of 80uM (levin et al. 1996). The studies also report that with higher concentration such as 2500 mg daily, the plasma levels of ascorbic acid could reach 100uM range (levin et al. 1996). The blood concentration of ascorbic acid depends upon its rate of absorption through the digestive tract and its rate of elimination by the kidney. Experiments was therefore planned around this value (100uM).
Ascorbic acid has been reported to function as an anticancer agent because of its antioxidant property through quenching of reactive oxygen species (ROS) and inhibit cell division and growth through production of hydrogen peroxide (Maramag et al: 1997). Ascorbic acid significantly affects cell growth and differentiation. It increases the survival time in cancer patients by 321 days (Cameron et al ; 1978). Multiple effects such as cytolysis, cell membrane disruption, mitochondrial alterations, nuclear and nucleolar reduction and increased phaolysosomes formation have been observed following ascorbic acid administration (Lupulescu 1992). It has also shown to positively modulate several genes such as fra-1, glutathione S-transferase Pi (GSTpi) and Mut L homologue-1 (MLH 1) in human cells (Catani et al; 2002). It exerts apoptotic effect on several leukemia cell lines, including HTLV-1 infected leukamia cells, by inducing expression of pro-apoptotic p53, p21, Bcl la genes and down regulating Bax expression (Harakeh et al; 2004a, b). Lower levels of ascorbic acid have been reported in cancer patients (Anthony and Schorah, 1982, Nunez et al; 1995, Kurbacher et al; 1996). Ascorbic acid is reported to be very cytotoxic to malignant cell lines (Koh et al; 1998. Roomi et al; 1998) and experts antimetastatic action (Liu et al; 2000). It also acts as a stabilizing agent for ECGC (Chen et al; 1998). 100uM concentration of ascorbic acid is physiologically achievable value.

4.2 Effect of graded concentration of L-lysine on MDA-MB 231, HT-1080 and HT-29

Table 3 Effect of L-lysine on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of L-lysine in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	200	+2	+4	+7
3	300	+13	-3	-2
4	400	+9	**-8.3**	**-45**
5	500	+22	**-22.3**	**-33**
6	600	+20	**-28**	**-33**

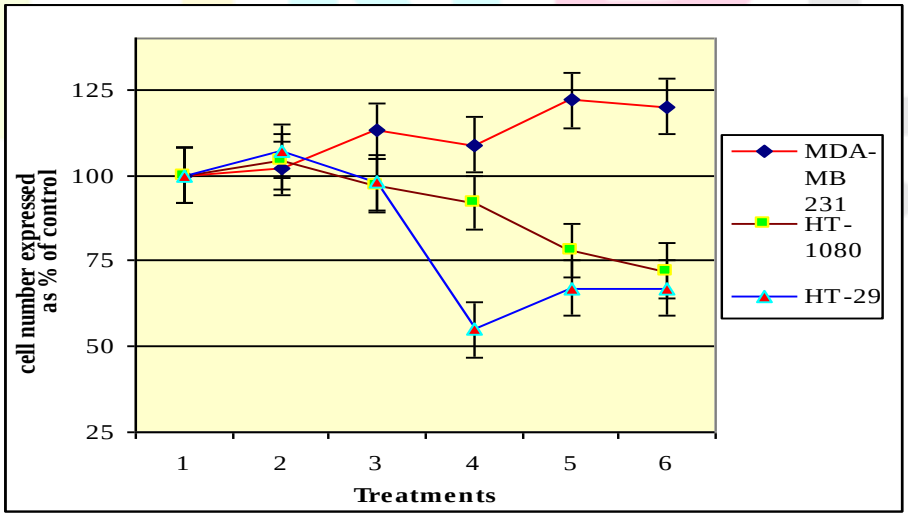

Figure 3: Effect of graded concentration of L-lysine on MDA-MB 231, HT-1080 and HT-29.

All the three cell lines show different behaviour to graded concentration of L-lysine (Table 3). For MDA-MB 231 L-lysine was proproliferative like ascorbic acid. At lowest concentration it showed proliferation of (+) 2% and highest proliferation of (+) 20% at highest concentration. Increase in the level of lysine caused significant increase in the cell proliferation uoto 300uM. Effect of 400uM, 500uM and 600uM did not differ significantly from that obtained with 300uM. HT-1080 and HT-29 were negatively affected especially at higher concentration, In HT-1080, the inhibition of growth was directly proportional to the concentration of L-lysine. Maximum inhibition of (-) 28% was obtained at 600uM in HT-1080. In HT-29 at 400uM there was (-) 45% inhibition and the results at 500uM and 600uM did not differ significantly from 400uM. The result obtained by 400uM to 600uM in both the cell lines i.e HT-1080 and HT-29 were significant.

In order for cancer cells to spread (metastasize) they have to penetrate the walls of blood vessels by dissolving collagen. The dissolving process requires special qnzyme, genetared/stimulated by the cancer cells, which bond to the collagen and dissolve it. L-lysine stops this process by attaching to the bonding sites preventing the enzyme attack. L-lysine helps inhibit metastasis by blocking the anchor site of collagenase enzyme. L-lysine given in higher dose is reported to be effective against cancer (Rath et al; 1992). The effect of L-lysine was different in different cell lines. For MDA-MB 231 it was proliferative, for HT-1080 and HT-29 it was inhibitory, but more pronounced in case of the later. 400uM level was selected as an ideal level.

4.3 Effect of graded concentration of L-proline on MDA-MB 231, HT-1080 and HT-29

Table 3 Effect of L-proline on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of L-proline in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	60	+0.3	***-8***	+7
3	100	+25	***-8***	***-22***
4	140	+30	+4	-5
5	180	+33	-5	***-40***
6	220	+30	+5	***-26***

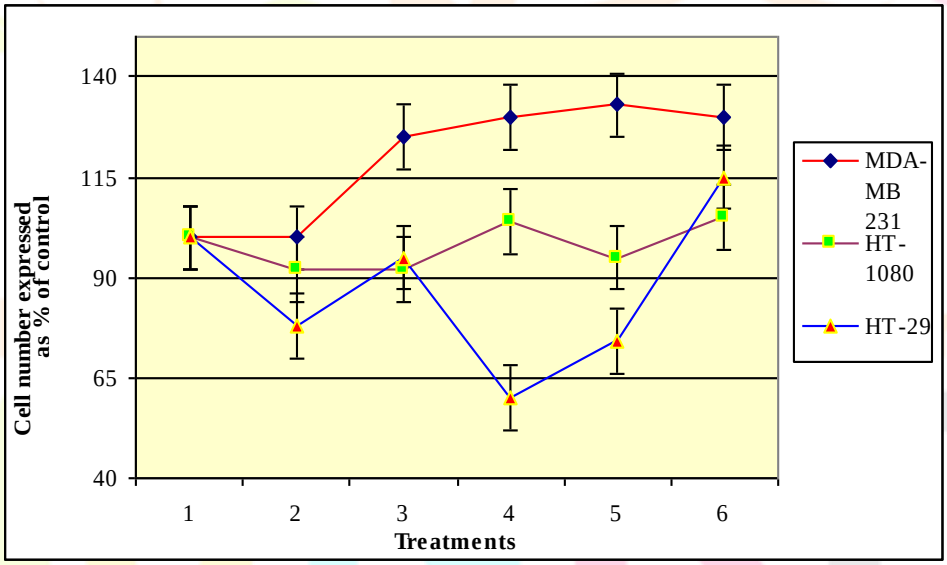

Figure 4: Effect of graded concentration of L-proline on MDA-MB 231, HT-1080 and HT-29.

L-proline at all concentrations (60uM, 100uM, 140uM, 180uM and 220uM) was proproliferative in case of MDA-MB 231. Significant increase in cell proliferation was obtained when the level of L-proline was raised to 100uM. Beyond that level there was no significant increase in cell proliferation upto 220uM of L-proline the highest level used in this experiment. In case of HT-1080, L-proline at lowest concentration was significantly antiproliferative. At other concentration the result was not significant. L-proline showed maximum cell inhibition of (-) 40% at 140uM in HT-29 cell lines and minimum inhibition of (-) 6% at 100uM. At 220uM the highest concentration cell proliferation was obtained of (+) 15%. The cell inhibition obtained at 60uM, 140uM and 180uM was significant when compared with control group. L-proline is normally synthesized in the body but its hydroxyproline content of tumor is reported to be low (Chubinskaia et al; 1989). Hydroxyproline excreted in the urine of cancer patients is also higher than that in healthy person (Okazaki et al; 1992). At 140uMm HT-29 was inhibited to maximum. In case of MDA-MB 231 and HT-1080, this level was proliferative.

Ascorbic acid (100uM), L-lysine (400uM) and L-proline (140uM) levels were also showed to have a significant antiproliferative and antimetastatic effect against some human cancer cell lines, breast (MDA-MB 231), colon (HCT116) and skin melanomas (A2058) (Netke et al; 2003).

4.4 Effect of graded concentration of L-arginine on MDA-MB 231, HT-1080 and HT-29

Table 3 Effect of L-arginine on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of L-arginine in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	200	+31	***-10***	***-33***
3	300	+15	***-27***	***-28***
4	400	+5	***-10***	***-21***
5	500	+13	***-24***	***-42***
6	600	+4	***-28***	***-34***

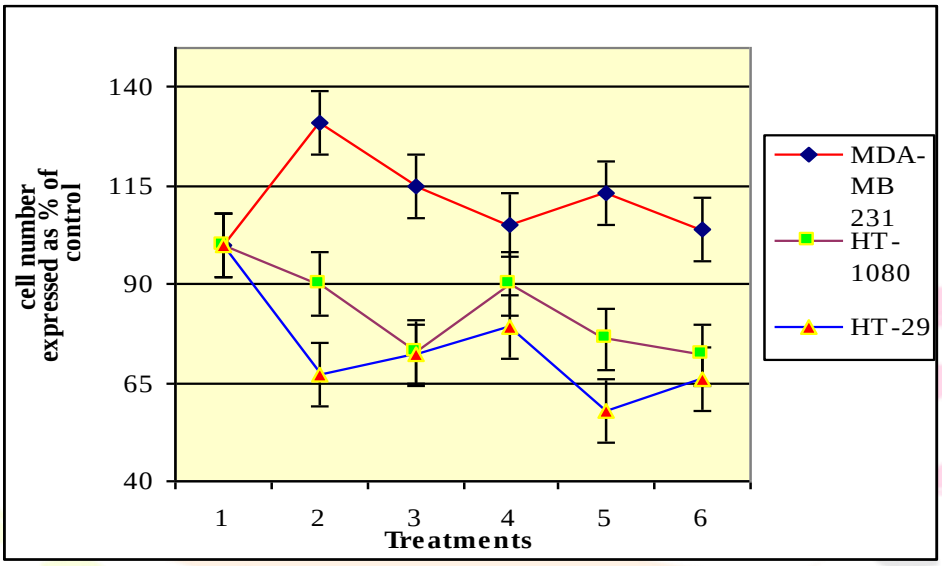

Figure 5: Effect of graded concentration of L-arginine on MDA-MB 231, HT-1080 and HT-29.

L-arginine showed bi-phasic effect in all the cell lines, but in MDA-MB 231 it was proliferative at all the concentration. The effect of L-arginine supplementation appears to be bimodal in MDA-MB 231. Supplementing the media with 200uM of L-arginine caused 31% increase in cell proliferation. However supplementation with 300uM resulted in only 15% increase in cell proliferation. The supplementation with 400uM and 600uM of L-arginine further reduced the increase in the cell proliferation to hardly 5%. Different concentration of L-arginine failed to obtained significant inhibition of MBA-MB 231.

L-arginine showed inhibitory effect on other cell lines. The inhibitory effect was more profound in HT-29 than HT-1080. L-arginine inhibition ranged between 10-28% for HT-1080 and 21-42% for HT-29. At 200uM of L-arginine, HT-1080 showed 10% inhibition and maximum inhibition of 28% was obtained at 600uM. In case of HT-29 at 400uM, 22% inhibition was obtained and at 500uM maximum inhibition of 42% was noted.

The cell inhibition obtained was not significantly different from each other at various concentrations but were significant when compared with there respective controls. Cooke et al; (1997) showed that any deficiency in arginine may limit the production of nitric oxide (NO) since it is a precursor of NO which acts as an inducer of apoptosis in breast cancer cell. Simeone et al; (2002) observed tat apoptotic agent such as phorbol esters, tumor necrosis factor-alpha (TNF-α), and peptide hormones increase NO production in breast cancer cell and the production of NO was directly correlated with the degree of apoptosis of these cells. The role played by NO in apoptosis of cancer cells was confirmed in nude mice xenograft of head and neck cancer cells by Kawakami et al; (2004). Netke et al (2003) showed that arginine acts synergistically with lysine in reducing Matrigel invasion. 400uM L-arginine showed moderate level of marginal proliferation for MDA-MB 231 while other cell lines showed inhibition.

4.4 Effect of graded concentration of N-acetyl cysteine on MDA-MB 231, HT-1080 and HT-29

Table 4 Effect of N-acetyl cysteine on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of L-proline in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	50	***-13***	***-5***	-2
3	75	***-19***	+10	***-33***
4	100	***-56***	***-33***	***-53***
5	125	***-71***	***-59***	***-72***
6	150	***-96***	***-85***	***-85***

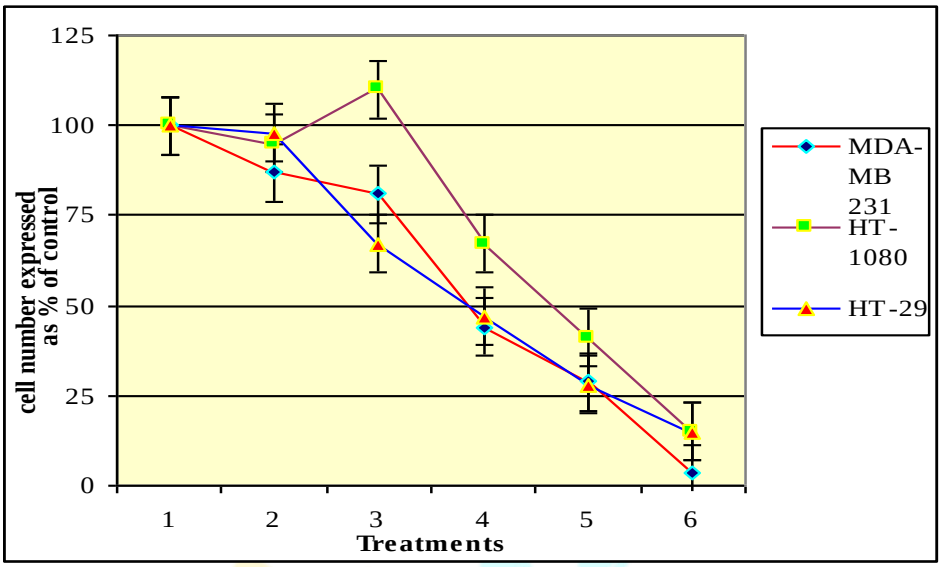

Figure 6: Effect of graded concentration of N acetyl cysteine on MDA-MB 231, HT-1080 and HT-29.

As the concentration increases the cell proliferation decreases. 96% cell inhibition was obtained with 150uM in case of MDA-MB 231. With 50uM concentration 13% cell inhibition was obtained and it was increased to 96% with rise in the concentration to 150uM. HT-1080 and HT-29 showed similar results. 5% cell inhibition was obtained with 50uM concentration and the inhibition range was increased as the concentration of NAC was increased. 75uM concentration showed 10% cell proliferation which was the only exception and was not significant, 100uMshowed 33% inhibition and 125uM showed 59% cell inhibition. At highest 150uM concentration 85% cell inhibition was obtained in case of HT-1080. Except for 75uM concentration rest of the concentration showed significant result. NAC in HT-29 showed significant results from 75uM concentration to 150uM. 33% cell inhibition was obtained by 75uM, 53% inhibition by 100uM, 72% inhibition by 125uM and 85% cell inhibition by 150uM of NAC.

NAC is a precursor of glutathione, a prominent antooxidant in the cell. NAC has shown promising results in cancer treatment. It has been reported to cause endothelial cell apoptosis and reduction in microvascular density (Agrawal et al; 2004). NAC showed anti-proliferative effect in all the three cell lines. A direct correlation between concentration and cell inhibition was observed for MDA-MB 231 and HT-29 but more pronounced effect was obtained in MDA-MB 231. This shows that NAC alone can be beneficial for the treatment of cancer. NAC is a thiol antioxidant precursor of glutathione (GSH) and also a mucolytic agent (Ventresca et al; 1989). NAC when used on pre-established tumors, showed sharp inhibition of tumor growth, with regression of tumors by 50% (Albini et al; 2001). In vitro studies showed NAC to be directly antomutagenic and anticarcinogenic; in vivo, NAC inhibited mutagenicity of a number of mutagenic materials, it also reduced the number of lung metastatic lesions (Morini et al; 1999), but considering the fact that many other nutrients help due to their antitumor or other bebeficial effects, it would be worth while to keep the concentration of NAC to minimum and try to find out whether spplementation of other nutrients act synergistically to bring down the proliferation. The minimum concentration of 50uM is also showing some cell inhibition, which can be increased by the addition of other nutrients to get maximum cell inhibition.

4.5 Effect of graded concentration of glutathione on MDA-MB 231, HT-1080 and HT-29

Table 5 Effect of glutathione on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of glutathione in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	50	***-28***	***-24***	***-29***
3	75	***-35***	***-26***	***-24***
4	100	***-33***	***-26***	***-48***
5	125	***-36***	***-29***	***-64***

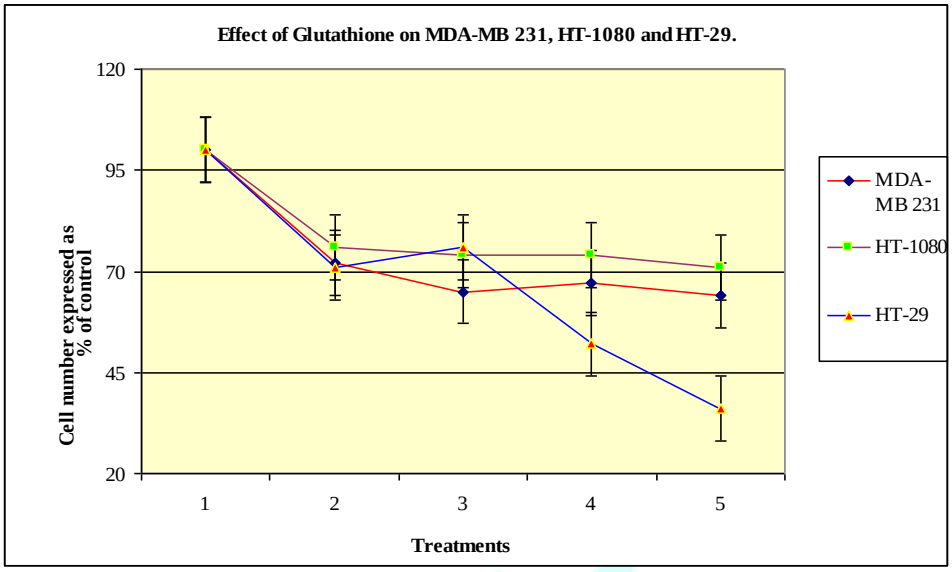

Figure 7: Effect of graded concentration of glutathione on MDA-MB 231, HT-1080 and HT-29.

The cytotoxicity of glutathione is compared for same concentartion as that of NAC. The effect of glutathione on cell proliferation of MDA-MB 231 was significantly different at each level of supplementation. At 50uM concentration it showed 28% cell inhibition and at 75uM it showed 35% cell inhibition. At this two concentration glutathione was significantly superior to NAC in respect of inhibiting the cell proliferation. At 100uM, 125uM and 150uM concentration 33%, 36% and 40% cell inhibition was seen respectively. At 150uM which is the highest concentration taken was not more efficient in inhibiting the cell proliferation as compared to NAC.

HT-1080 showed 20% to 30% cell inhibition for all the concentration. There was no difference in cell inhibition with increase in concentration. At 50uM concentration 24% cell inhibition was obtained and at 75uM, 100uM cell inhibition obtained was 26% where as at 125uM concentration 29% cell inhibition was seen. At 150uM the highest concentration used the cell inhibition was 32%. All the values obtained were significant against control group.

Same was the result obtained with HT-29. Glutathione at higher concentration was more efficient in inhibiting the cell growth. At 125uM it showed 64% cell inhibition and at 50uM only 29% cell inhibition was obtained. It shows different results with different cell lines and all showed significant result.

Glutathione plays a variety of physiological roles, including regulation of signal transduction (Shreck 1991), intracellular defense against oxidative stress (Meister 1994), and other functions important in the neoplastic process. The effect of NAC and glutathione on cell proliferation was significantly different at each level of supplementation. Glutathione was significantly superior to NAC at 50uM and 75uM in respect of inhibition of cell proliferation. While at higher concentration NAC was more efficient in inhibiting the cell proliferation.

4.6 Effect of graded concentration of Selenium on MDA-MB 231, HT-1080 and HT-29

Table 6 Effect of Selenium on MDA-MB 231, HT-1080 and HT-29

Sr No.	Concentration of Selenium in uM	% Inhibition against control of MDA-MB 231	% Inhibition against control of HT-1080	% Inhibition against control of HT-29
1	Control	0.0	0.0	0.0
2	3	***+5***	***-8***	-1
3	4	***-45***	***-41***	***-31***
4	5	***-60***	***-48***	***-51***
5	6	***-79***	***-58***	***-52***
6	7	***-83***	***-67***	***-58***

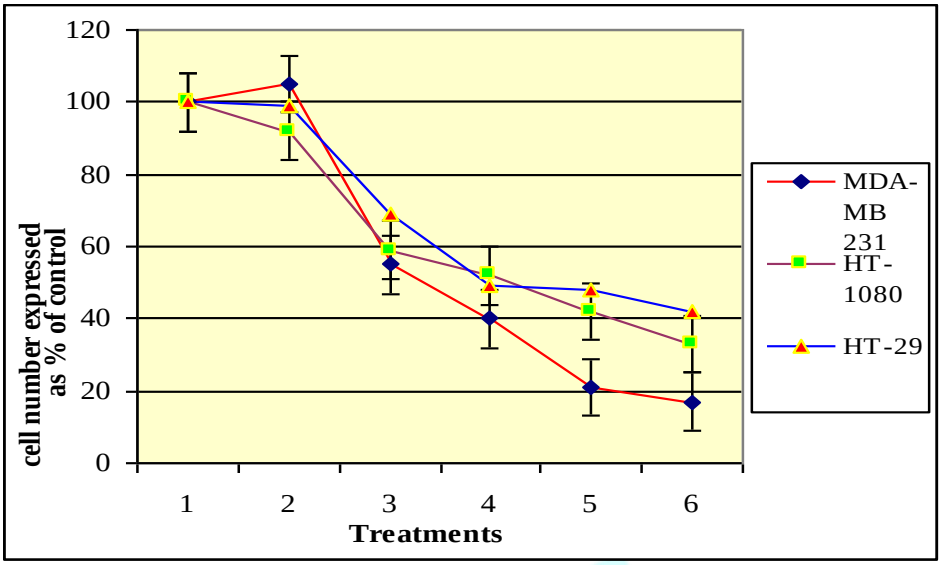

Figure 7: Effect of graded concentration of Selenium on MDA-MB 231, HT-1080 and HT-29.

Selenium is neurotoxic hence our aim was to find out the response of cell lines to minimum concentrations of selenium. There was 5% increase in cell proliferation with 3uM selenium in MDA-MB 231. This increase was not significant. As the concentration of selenium was increased the cell proliferation decreased. With 4uM concentration 45% cell inhibition was obtained and as the concentration was increased cell inhibition also increased and with 7uM the highest concentration tried in experiment the cell inhibition obtained was also highest of 83%. The result obtained by all the concentration except 3uM was significant and with the increase in concentration cell inhibition also increases. The results obtained with HT-1080 and HT-29 was matching, with the minimum concentration i.e. 3uM selenium the cell inhibition was minimum of 8% and 1% respectively. The cell inhibition obtained with 4uM to 7uM concentrations was from 31% to 67%. HT-1080 showed 41% cell inhibition with 4uM. 48% inhibition with 5uM selenium, 58% inhibition with 6uM concentration and 67% cell inhibition with 7uM selenium concentration. In HT-1080 as the concentration was increased there was some rise in cell inhibition. All the concentration showed significant results. But this was not the case with HT-29. After 4uM concentration with increase in concentration there was no rise in cell inhibition. 4uM selenium showed 31% cell inhibition and 5uM, 6uM and 7uM selenium showed 51%, 52% and 58% cell inhibition.

The trace mineral selenium is not itself an antioxidant, but within cells it is incorporated into selenoproteins, some of which have antioxidant functions (i.e. glutathione peroxidase). Selenium directly induces tumor cell apoptosis and inhibits their spread in the tissue. It inhibited matrigel invasion by HT-1080 fibrosarcoma cells and reduced the production of MMP2 and MMP9 by fibrosarcoma cells (Yoon et al; 2001). Selenium interfered with the activity of MMP9 and reduced the migration of endothelial cells through ECM (Tosetti et al; 2002 and Morini et al; 1999). Selenium is very neurotoxic and at higher concentration it may affect the normal cell, our effort was to use minimum level of selenium that would bring about the desired effect by combining it with other nutrients.

The response of all the three cell lines was not the same to graded concentrations of ascorbic acid. It was pro poliferative for MDA-MB 231 at all the levels while HT 1080 and HT 29 showed bi-phasic effect. At 50, 125 and 150uM, it was significantly anti-proliferative. HT 29 showed positive proliferation at 75 and 100uM.

L-Lysine at all concentrations, (100, 200, 300, 400 and 500uM) was pro-proliferative for MDA-MB-231, but HT 1080 and HT 29 was affected adversely especially at higher concentrations.

L-Proline at all concentrations (60, 100, 140, 180 and 220uM) was pro-proliferative but for other two cell lines it showed biphasic effect. L-Arginine (100, 200, 300, 400, and 500uM) showed bi-phasic effect in all the three cell lines, but in MDA-MB-231 it was more or less proliferative. N-acetyl cystein and glutathione were antiproliferative at the concentrations of 50, 75, 100, 125 and 150uM with an exception of 50uM NAC having positive effect on the growth of HT 29.

L-Proline is normally synthesized in the body but its hydroxyproline content of tumor is low. (Chubinskaia et al, 1989). The body does not synthesize L-Lysine, another essential amino acid. Decrease food intake of cancer patients results in deficiency of essential amino acids. L-Arginine is a conditionally essential amino acid. N-acetyl cystein supplies bioavailable cystein necessary for replenishment of glutathione (GSH) that plays a variety of physiological roles, including regulation of signal transduction (Shreck 1991), intracellular defense against oxidative stress (Meister 1994) and other functions important in the neoplastic process. NAC down regulates the expression of insulin like growth factor receptor (IGF-IR) in colon cancer cells. NAC at 40uM level in cell culture studies reduced the cell proliferation of colon cancer cells to 20% (Kelly et al 2002). NAC was able to reduce the invasive and metastatic potential of melanoma cells and inhibit tissue invasion of endothelial cells (Tosetti et al 2002). There are reports, which show that the amino acids are able to stop the cell proliferation of cancer, invasion of ECM (metastasis), induction of apoptosis and reduction of angiogenesis.

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