TED ANKARA COLLEGE FOUNDATION

PRIVATE HIGH SCHOOL

Biology Extended Essay

Investigating the cytotoxic effect of milk powder

on U-118 MG human glioblastoma cell series

Supervisor's Name: Sevim Saral

Name of the Candidate: Alper Taş

Candidate Number: D001129-0040

Word Count: 3997

Abstract

The purpose of my extended essay is to observe the cytotoxic effect of milk on the human glioblastoma cells. In order to reach this purpose, different concentrations of milk powder solutions are prepared and the inverse proportion between the concentration of the milk powder solutions and the viability of the cells is shown.

The research question is "Does milk powder cause a cytotoxic effect on U-118 MG Human Glioblastoma Cells; does the cytotoxic effect that milk powder causes on human glioblastoma cells increase as the concentration of the milk powder solution increases?"

It is hypothesized that; there will be a significant mean difference between the number of human glioblastoma cells when the concentration of milk powder solution is increased.

In order to search the validity of the hypothesis, U-118 MG Human Glioblastoma Cell Series are used. These cells are resolved in a medium with 90% RPMI with L-glutamine, %10 Fetal Bovine Serum and 1% antibiotics. For the passaging procedure Trypsin-EDTA solution is used. By using the counting chamber cell concentrations which contains 20.000 cells in 200 µL of medium is prepared. Then milk powder solutions with the concentrations of 10, 20, 40, 80 100 mg/ml and a control group in which no milk powder solution is added are prepared. Five trails are made for each group and by using MTT Assay, cell viability is measured for the 24^{th} hour. Statistical analyse is done to show the decrease in the viability of the cells.

The data collected with the MTT assay is processed and the mean of the trials for each group is calculated. By using t-Test, the significant decrease in the viability of the cells is found. Although the decrease for the concentration 10 mg/ml solution of milk powder isn't significant, the decrease for the concentrations of 20, 40, 80 and 100 mg/ml solutions of milk

powder is significant since the p values for them are smaller than 0.05. By using Anova Statistics, it is seen that there is a significant mean difference between the absorbance values of solutions which contain different concentration of milk powder. As a result of the experiment, it is found out that the milk powder has a cytotoxic effect on the glioblastoma cells and the number of the cancer cells decrease as the concentration of the milk powder solution is increased.

Word Count: 386 words

Content

Introduction and Background Information:

Cancer, which is also called as malignant neoplasm, is a type of diseases which is caused due to the unregulated growth of the cells. It can also pass to the other vital parts of the body by way of lymphatic system and blood stream. There exist more than 200 various kinds of cancers that cause great damages in human body. [1]

Nowadays, cancer is one of the most commonly encountered diseases that causes death. It is said that number of people who will be dealing with cancer will increase in time and this makes people frightened. Cancer can be healed by surgery, chemotherapy, radiation therapy, immunotherapy, and monoclonal antibody therapy. The selection of therapy is related with position and degree of the tumor and phase of the disease, besides the overall status of the patient. Also, some new ways for the treatment are being searched for by scientists, because these treatments have some side effects, and they are not always effective to clean the body completely from cancer cells. [2]

Cancer has a lot of types such as colon, lung, kidney, pancreatic, thyroid and prostate cancer.[3] Brain tumor is one of the most common and dangerous types of cancer. Glioblastoma multiforme (GBM) is the most frequent and dangerous of malignant primary brain tumors in grown ups and is one of a group of tumors referred to as gliomas. GBM is formed mainly in cerebral hemispheres, besides it can be formed in other parts of the brain, brainstem and spinal cord. There is not a certain cause for this disease, but recent researches show that genetic mutations play a role in the occurrence of it. Glioblastoma

[1] http://en.wikipedia.org/wiki/Cancer
[2] http://en.wikipedia.org/wiki/Management_of_cancer
[3] http://www.cancer.gov/cancertopics/types/commoncancers

multiforme is seen generally in old people and men have this disease more often than women.[4]

Focusing on the purpose of improving patient's lives, scientists made researches about the negative or positive effects of some foods. Milk is one of these foods which is thought to be increasing the number of cancer cells in a cell line.[5] Today, it causes confusion in people's mind. People think that milk is a natural and a beneficial product. So, they ask themselves how such a salutary food can effect breast cancer cells negatively. Since people are giving a great importance to their health, the ideas about the effects of milk find a wide range of place in media, but still there is not very clear information.

There are many studies related with the effects of milk on cancer cells. However, results are contradictory with each other. The reason for the difference between these results can be shown as that milk includes both beneficial and harmful components.

Ingredients of milk, such as calcium, vitamin D, stearate, lactaptin, have been found to stimulate the apoptosis of breast cancer cells. It can be said that milk has been found to be protective against breast cancer when drank in babyhood and childhood.

On the other hand, drinking milk in adulthood can be risky. One investigation of rats which have carcinogen-induced mammary tumours supported that while taking the ovaries of rats out decreased the number and size of the tumours, feeding milk to similar ovariectomized rats led to raises in mammary tumour occurrence, tumour number and tumour volume. Intake of either nonfat or whole milk also has been found to enhance the occurrence and volume of tumours in experimental rats which have carcinogen-induced

[4] http://www.braintumour.ca/4869/glioblastoma-multiforme
[5] http://foodforbreastcancer.com/foods/milk

mammary tumours. In addition, high consumption of animal fats has been associated in some investigations with enhanced breast density, a risk agent for breast cancer and recrudescence. [6]

In a study; recombinant Bovine Growth Hormone (rBGH) is syringed to cows and it is seen that milk of these cows increases risks of breast and colon cancers in humans. The research supports that rBGH raises the amount of insulin-like growth factor (IGF-1) in milk. IGF-1 is a strong trigger and controller for the growth and division of the cells in humans and cows. The research indicates that enhanced levels of IGF-1 pose danger for breast cancer and colon cancer.[7]

IGF-1 can't be get rid of by pasteurization. Actually, this process significantly enhances the amount of IGF-1 in milk. IGF-1 also can't be cleared off by digestion. In addition, Food and Drug Administration (FDA) approves that IGF-1 is readily absorbed along the intestinal wall. Moreover, last research demonstrates that IGF-1 can be absorbed into the bloodstream and by this way, it can influence other hormones.[8]

IGF-1 stimulates quick division and reproduction of healthy human breast epithelial cells in tissue cultures. It is most probably that IGF- 1 encourages conversion of healthy breast epithelium to breast cancer cells. In addition, IGF-1 sustains the malignancy of human breast cancer cells and tumour can spread to other organs. [9]

However, there aren't many experiments which are done about the effect of milk on glioblastoma cells. But one of the studies says that calcium may produce a protective impact

[6] http://foodforbreastcancer.com/foods/milk
[7] http://www.preventcancer.com/avoidable/colon_cancer/milk_colon.htm
[8] http://www.preventcancer.com/avoidable/colon_cancer/milk_colon.htm
[9] http://www.preventcancer.com/avoidable/colon_cancer/milk_colon.htm

against gliomas by stimulation of apoptosis, DNA repair, or other procedures. A comparison of the effect of intake of calcium and other dairy ingredients and dairy foods between 337 people who are dealing with glioma and 450 healthy people showed that there is a remarkable inverse relation between calcium consumption and glioma occurrence for women.[10]

Main notion of this study is to analyse the effect of milk powder on the glioblastoma cancer cells and to observe whether there is an important change in the number of the glioblastoma cells. Glioblastoma cells are obtained from Ankara University Medical Biology Laboratories. These cells were produced and hold in proper conditions for use in cancer researches. Different concentrations of milk powder are prepared and the change in the number of glioblastoma cells is investigated.

Cytotoxicity is the quality of being toxic to cells. An immune cell or some types of venom (e.g. from the puff adder or brown recluse spider) are the example of toxic agents. Treating cells with the cytotoxic compound can cause cell deaths in some different ways.[11] In the treatment of cancer disease cytotoxic agents are used. So, in this study, cytotoxic effect of milk powder is investigated.

[10] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047463/
[11] http://en.wikipedia.org/wiki/Cytotoxicity

Hypothesis:

Milk is a product which is produced by animals such as cow or goat. It is consumed very often by many people since old times. According to the common belief of people, milk is a beneficial product and it can be good for some diseases. Some researches show that milk increase the number of cancer cells and it is not recommended for patients. But some researches show that milk is beneficial for the body defence. Especially the breast milk is seen as an important nutrition type for small babies. In a study it is explained that milk reduces the mammary tumour size because of the components such as vitamin D and lactoferrin in it.[12]

Milk can not be used directly to heal the cancer cells but it can be helpful to curing process. For example, in a study it is shown that camel milk stimulates apoptotic signaling pathways in human hepatoma and breast cancer cell lines by the transcriptional procedure.[13]

Although, milk is found harmful for patients who deal with breast or colon cancer, there isn't a significant study related with the impact of milk on glioblastoma cells. However, in a study it is explained that the intake of dairy products can decrease the incidence of glioma for women.[14] Concentrating on this study it is hypothesized that, the number of human glioblastoma cells will decrease when the concentration of milk powder is increased, so there will be an inverse proportion between the number of human glioblastoma cells and the concentration of the milk powder.

[12] http://foodforbreastcancer.com/foods/milk
[13] http://www.ncbi.nlm.nih.gov/pubmed/22654482
[14] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047463/

This study can give some idea about the effect of milk on cancer cells. However, the information is limited since the experiment is only done on U-118 MG cell series (human glioblastoma cells) in in-vitro conditions.

Method Development and Planning:

In this investigation, MTT assay (See Appendix A for the general information about MTT Assay) is used for measuring the viability of the glioblastoma cancer cells. The use of MTT assay provides much accurate and reliable result than other methods for counting cells, because the measurement for determining the cell viability is done by using the absorbance values of the waves captured by the living cells. The procedure is mainly focused on recording the absorbance values measured by the spectrophotometer for the absorbance values of OD_{550}-OD_{690} and calculating the values by using MTT assay. [15]

By using the MTT assay method, human caused random errors will be prevented since the assay calculates the absorbance of the solution. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means and the absorbance value becomes high, but if the MTT Reagent yields low background absorbance values then the cells are lack in number or absent.[16] In addition, it will be easy to analyse the results of the experiment and the results will be much accurate. Because of these reasons MTT assay is a reliable method to calculate cell viability.

MTT assay is used in many studies which includes cell viability calculation. Since this experiment is also related with the cell viability, the MTT assay can be used in this experiment. The chemical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide will be used as a reagent for the MTT assay. There is not any source that finds this chemical

[15] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"
[16] http://www.atcc.org/~/media/DA5285A1F52C414E864C966FD78C9A79.ashx

inappropriate for the experiment and it is widely used in such type of studies. So, there is no problem related with the use of method and the types of the chemicals.[17]

In this experiment the type of the substance that will affect the cell viability is chosen as milk powder. There couldn't be found a research which investigates directly the effect of milk powder on glioblastoma cancer cells or other cancer cells. However, in a study it is explained that the intake of dairy products can decrease the incidence of glioma for women.[18] Since milk powder is a dairy product, it is hypothesised that milk powder has a cytotoxic effect on the glioblastoma cells and it causes a decline in the number of the cells. When the metabolic activity of the cells are stopped or slowed down, the yellow tetrazolium MTT[19] won't be reduced completely and the colour of the final solution won't be as purple as it is in metabolically active cells. Then the absorbance of this solution will decrease and it will give an idea about the viability of the cells.

The milk powder that will be used in the experiment is bought from a market and it is chosen to be a common and unadulterated one in order to prevent another substance in the product to affect the viability of cells and generalise the result for the entire milk powder. U-118 MG cells are used in this study in order to examine the effect of milk powder on the cell viability. Since these cell series are ready for the experiments in Ankara University Laboratory, they are chosen for the experiment.

The concentration interval of milk powder is estimated by making trials and examining the similar studies. U-118 MG cell series are grown in proper medium in which substances that are required for the cell growth is found.

[17] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"
[18] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047463/
[19] http://www.atcc.org/~/media/DA5285A1F52C414E864C966FD78C9A79.ashx

For each trial same number of glioblastoma cancer cells are collected by using the Neubauer Counting Chamber (See Appendix B for the general information about Neubauer Counting Chamber). Cells are incubated at 37°C under a 5% CO_2 humidified incubator. In order keep the external factors constant Biosafety Cabinet is used.

It is expected that as the concentration of the milk powder is increased, the cell viability values measured by MTT assay will decrease and the maximum cell viability will be measured in the control group in which no milk powder is added.

Apparatus for the experiment: [20]

200 gram PINAR milk powder

U-118 MG Human Glioblastoma Cell Series

Incubator

Laboratory Centrifuge

Neubauer Hemocytometer

Liquid Nitrogen Storage

MTT assay

Biosafety Cabinet

96 welled plate

SOFT (Spectrophotometer Technology) Programme

15 cm^3 and 50 cm^3 falcon tube

T-75 flask

Pipette

Filter funnel

Gloves

Galosh

[20] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"

Bonnet Mask

Ethanol

Apparatus for medium preparation: [21]

RPMI with L-glutamine (90%)

Fetal Bovine Serum (10%)

Antibiotics (1.000.000 U/ml penicillin and 1 gram/ml streptomycin) (1%)

>> All of the equipment which is essential for the experiment is provided by the Medical Biology Laboratory of Ankara University.

[21] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"

Method:

Before the experiment starts; bonnet, gloves and galosh should be dressed. The sterilization of the apparatus should be done by using ethanol solution.

Resolving U-118 MG cell series in the medium [22]

Make the medium ready with 90% RPMI with L-glutamine, 10% Fetal Bovine Serum and 1% antibiotics.

Put 5 cm^3 of medium into the falcon tube.

Take out the U-118 MG cell series out of the nitrogen storage and put these cells into the falcon tube which has a volume of 15 cm^3.

Blend the medium with the U-118 MG cells.

Centrifuge the falcon tube at the speed of 1500 rpm during 2 minutes.

Take out the supernatant, the part that remains on top of the cells later than the centrifuge process is completed.

Put 20 cm^3 of new medium and mix the tube and transfer the cells to the T-75 flask.

Incubate at 37°C under a 5% CO_2 humidified incubator.

Passaging Procedure

Wash the cells with the Phosphate Buffer Saline (PBS).

[22] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"

Put Trypsin-EDTA solution into the culture dish. Scatter the solution completely above the surface of the cells.

Incubate the dish in the cell culture incubator (37°C) during 2 or 3 minutes. Wait and look at the cell morphology (shape of cells) very carefully under the microscope. If the cells begin to seem rounded, softly tap the flask to take out the cells entirely from the surface. Unless the cells are removing easily from the substratum after the soft tapping, continue to incubate for some more time in the incubator.

Wash out whole the cells from the surface by pipetting the fresh complete culture medium (5 ml) above the entire surface. Pipette softly 4 or 5 times to break cell clusters.

Keep the flask in the cell culture incubator. [23]

Experiment: [24]

Transfer the cells from the flask to the 50 cm^3 falcon.

Centrifuge the falcon in 1000 rpm during 2 minutes.

Remove the supernatant.

Add 20 cm^3 of fresh medium.

Use hemocytometer to measure the number of the cells.

Dilute the medium in order to get 200.000 cells in 20 cm^3 medium.

[23] http://bioinfoweb.com/Protocol-passaging-of-adherent-cell-culture-using-trypsin-EDTA.htm
[24] Elif Deniz Oğuz, "Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)"

Put 200 µL of medium with U-118 MG cells to each well in a 96 welled plate in order to make every trial with 20.000 cells

Prepare 100, 80, 40, 20 and 10 mg/ml concentrations of milk powder solution and pour each of them to the 96 welled plate.

Incubate U-118 MG cell line at 37 °C under a 5% CO_2 humidified incubator.

MTT assay

Twenty-four hours after incubating the cells with the estimated concentrations of milk powder solution in a 96-well cell culture plate at 37°C under a 5% CO_2 humidified incubator, take a 96 welled plate and add 10 µL of MTT reagent to 30 of the wells, since there are 6 groups and 5 trials for each group.

Mix the cells with the MTT reagent by pipetting.

After 4 hours put 100 µL of solubilisation solution.

Incubate the cells at 37 °C under a 5% CO_2 humidified incubator for 12 hours.

With the help of spectrophotometer measure OD_{550} and OD_{690} absorbances.

Record the data and interpret the results.

Results:

The table is formed according to the results measured by the MTT assay that is used for the determination of cell viability. The data for the absorbance values of OD_{550}-OD_{690} is processed as it is estimated in the procedure of MTT assay.

The table is formed by calculating the average absorbance value of five trials for each concentration of milk powder.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
0 mg/ml milk powder solution	Trial 1	2.365	0.072
	Trial 2	2.184	0.092
	Trial 3	2.251	0.068
	Trial 4	2.334	0.068
	Trial 5	2.374	0.073

Table 1: The absorbance values measured with the MTT assay cell proliferation kit for trials of 0 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550-690}$ after 24 hours (Optical Density - OD)
0 mg/ml milk powder solution	Trial 1	2.293
	Trial 2	2.092
	Trial 3	2.183
	Trial 4	2.266
	Trial 5	2.301

Table 2: The value of $OD_{550-690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 0 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
10 mg/ml milk powder solution	Trial 1	2.098	0.073
	Trial 2	2.038	0.074
	Trial 3	2.168	0.070
	Trial 4	2.392	0.074
	Trial 5	2.303	0.069

Table 3: The absorbance values measured with the MTT assay cell proliferation kit for trials of 10 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550\text{-}690}$ after 24 hours (Optical Density - OD)
10 mg/ml milk powder solution	Trial 1	2.025
	Trial 2	1.964
	Trial 3	2.098
	Trial 4	2.318
	Trial 5	2.234

Table 4: The value of $OD_{550\text{-}690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 10 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
20 mg/ml milk powder solution	Trial 1	2.074	0.073
	Trial 2	2.098	0.074
	Trial 3	2.114	0.073
	Trial 4	1.918	0.068
	Trial 5	1.972	0.070

Table 5: The absorbance values measured with the MTT assay cell proliferation kit for trials of 20 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550-690}$ after 24 hours (Optical Density - OD)
20 mg/ml milk powder solution	Trial 1	2.001
	Trial 2	2.024
	Trial 3	2.041
	Trial 4	1.850
	Trial 5	1.902

Table 6: The value of $OD_{550-690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 20 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
40 mg/ml milk powder solution	Trial 1	1.594	0.065
	Trial 2	1.401	0.063
	Trial 3	1.209	0.062
	Trial 4	1.395	0.065
	Trial 5	1.547	0.065

Table 7: The absorbance values measured with the MTT assay cell proliferation kit for trials of 40 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550-690}$ after 24 hours (Optical Density - OD)
40 mg/ml milk powder solution	Trial 1	1.529
	Trial 2	1.338
	Trial 3	1.147
	Trial 4	1.330
	Trial 5	1.482

Table 8: The value of $OD_{550-690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 40 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
80 mg/ml milk powder solution	Trial 1	0.121	0.058
	Trial 2	0.115	0.056
	Trial 3	0.102	0.057
	Trial 4	0.103	0.061
	Trial 5	0.151	0.091

Table 9: The absorbance values measured with the MTT assay cell proliferation kit for trials of 80 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550-690}$ after 24 hours (Optical Density - OD)
80 mg/ml milk powder solution	Trial 1	0.063
	Trial 2	0.059
	Trial 3	0.045
	Trial 4	0.042
	Trial 5	0.060

Table 10: The value of $OD_{550-690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 80 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		Absorbance values after 24 hours (Optical Density - OD) (±0.001)	
		OD_{550}	OD_{690}
100 mg/ml milk powder solution	Trial 1	0.116	0.061
	Trial 2	0.115	0.054
	Trial 3	0.164	0.065
	Trial 4	0.138	0.078
	Trial 5	0.198	0.092

Table 11: The absorbance values measured with the MTT assay cell proliferation kit for trials of 100 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

		$OD_{550-690}$ after 24 hours (Optical Density - OD)
100 mg/ml milk powder solution	Trial 1	0.055
	Trial 2	0.061
	Trial 3	0.099
	Trial 4	0.060
	Trial 5	0.106

Table 12: The value of $OD_{550-690}$ which is calculated according to OD_{550} and OD_{690} values recorded by the MTT assay cell proliferation test procedure for the trials of 100 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

Concentration of the milk powder solution (mg/ml)	**Average absorbance value recorded by the MTT assay for the 24th hour (Optical Density - OD)**
0	2.227
10	2.128
20	1.964
40	1.365
80	0.054
100	0.076

Table 13: The average absorbance values are calculated according to the MTT assay cell proliferation kit for the trials of 0, 10, 20, 40, 80 and 100 mg/ml of milk powder solution added to U-118 Mg cell series for 24th hours.

Data Analysis:

Statistical Analysis:

The computer program Microsoft Word Excel 2013 is used in order to obtain the descriptive statistics, p values according to the t-Test and p value according to Anova statistics. In this study mean, standard error, standard deviation and confidence level (95,0%) are calculated as part of a descriptive statistics and t-Test is done in order to determine whether there is a significant change between the mean values of the two groups (absorbance values in the control group and absorbance values in one of the groups that milk powder is added). In addition, p value calculated by Anova makes a multiple comparison and determines either there is a significant change between the mean values of the multiple groups. If the p-value is smaller than 0.05 the null hypothesis is rejected. So, it means that there is a significant change between the groups.

	Concentration of milk powder solutions (mg/ml)					
	0	*10*	*20*	*40*	*80*	*100*
Mean	2.227	2.128	1.964	1.365	0.054	0.076
Standard Error	0.040	0.066	0.037	0.067	0.004	0.011
Standard Deviation	0.089	0.146	0.083	0.150	0.010	0.024
Confidence Level(95,0%)	0.110	0.182	0.103	0.186	0.012	0.030

Table 7: Descriptive statistical table which shows the mean, standard error, standard deviation and confidence level (95,0%) values for the milk powder solutions with concentrations of 0,10, 20, 40, 80 and 100 mg/ml

Concentration of the milk powder solution (mg/ml)	**p values calculated by t-Test (control group versus the milk powder added trials)**
10	0.231
20	0.001
40	4.000×10^{-6}
80	1.439×10^{-11}
100	1.989×10^{-11}

Table 8: p values calculated by t-Test which will show whether the results are significant for the trials with the 10, 20, 40, 80 and 100 mg/ml milk powder solutions.

ANOVA

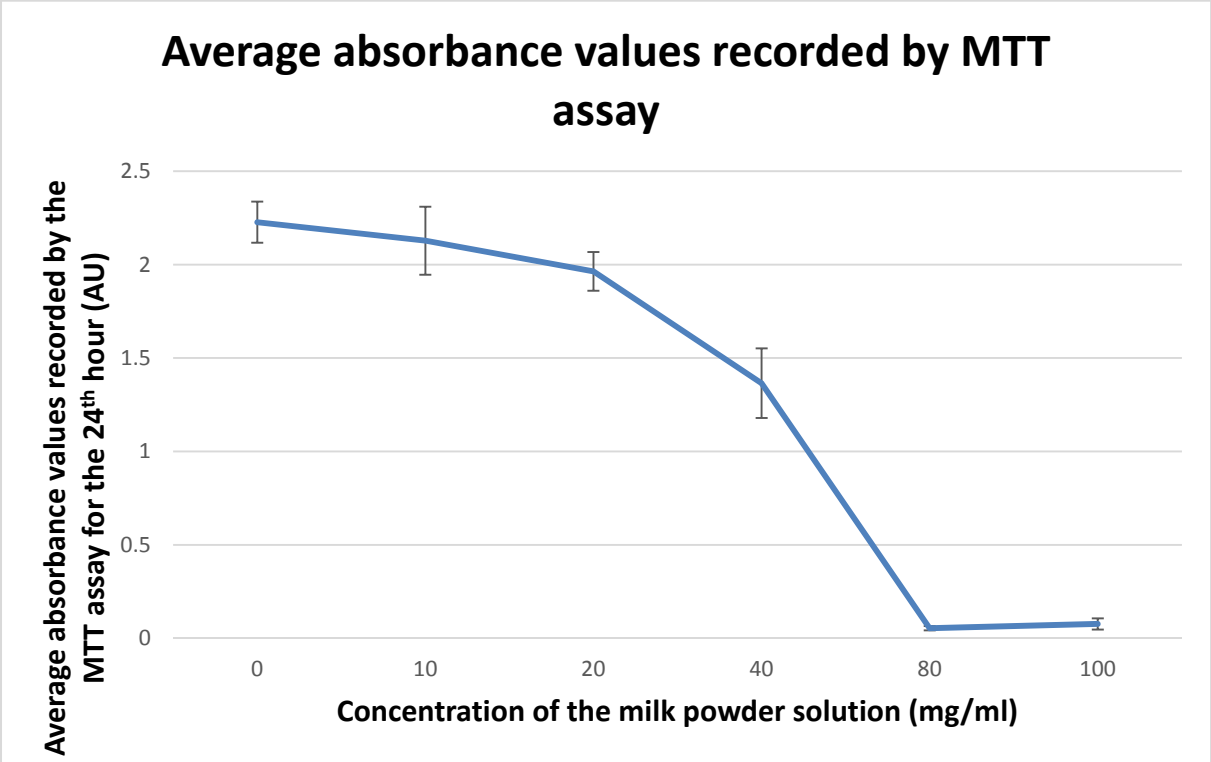
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25,19933947	5	5,039867893	508,7084516	**1,59284E-23**	2,620654148
Within Groups	0,2377724	24	0,009907183			
Total	25,43711187	29				

Table 9: Anova statistical table which shows the p-value for the mean absorbance values of the in the groups in which are 10, 20, 40, 80 and 100 mg/ml milk powder solutions are added.

For the comparison of the mean viability of cells in the control group and the group in which milk powder solution with concentration of 10 mg/ml is added, p value calculated by t-Test is 0.231 and it is bigger than 0.05, so it means that there isn't a significant difference between the viability of the cells in the control group and that is in 10 mg/ml solution of milk powder.

For the comparison of the mean viability of cells between the control group and the groups in which milk powder solution with concentration of 20, 40, 80 and 100 mg/ml are added, p values calculated by t-Test are smaller than 0.05, so it means that there is a significant difference between the viability of the cells in the control group and that are in 20, 40, 80 and 100 mg/ml solutions of milk powder.

Data Analysis in a Graphical Sense:



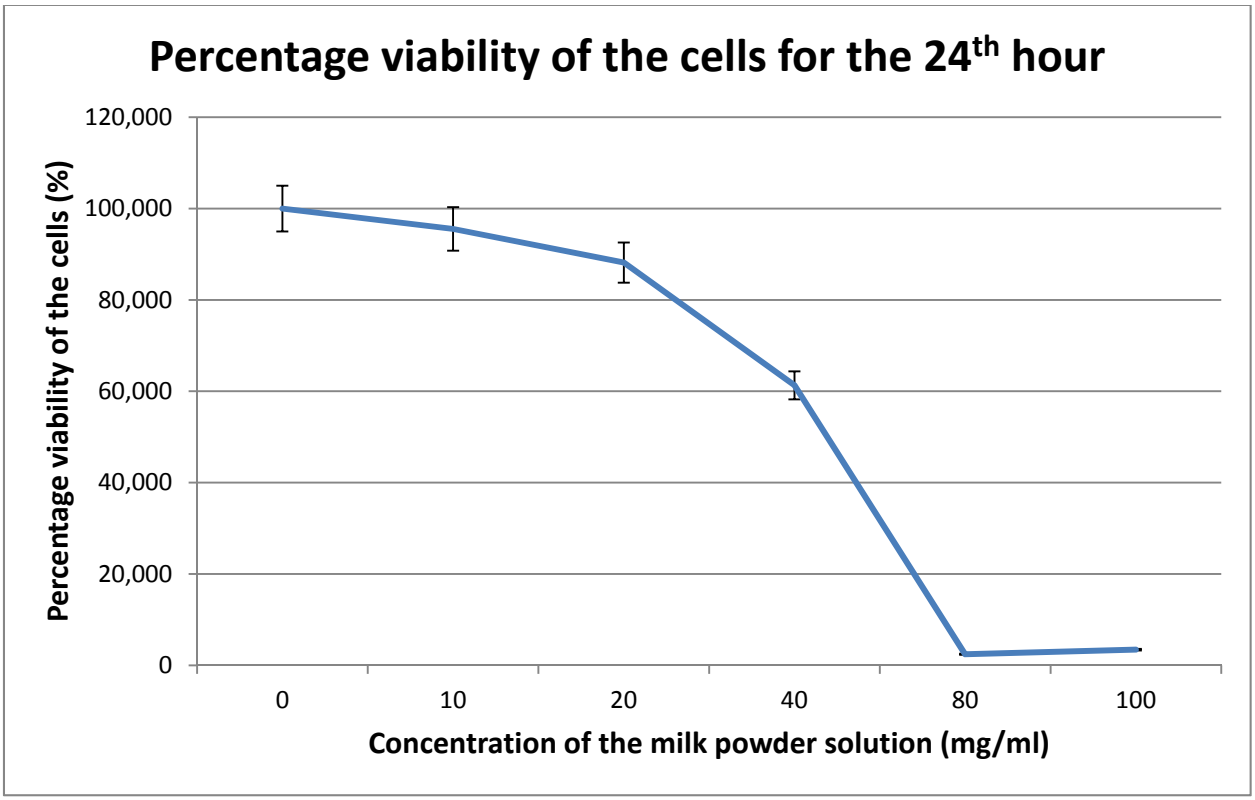
Graph 1: Graph of the comparison of the cell viability of U-118 Mg cell series in 0, 10, 20, 40, 80 and 100 mg/ml concentrations of milk powder solution for 24th hours. The absorbance values are recorded by MTT assay kit and the graph is drawn according to the average values of the absorbance

Percentage Calculation:

In order to see the change in the viability of the U-118 Mg cell series much obviously in different concentrations of milk powder solutions percentage calculation is done. The percentage viability of the cells when the concentration of the milk powder solution is 0% is chosen as 100%.

Concentration of the milk powder solution (mg/ml)	**Percentage viability of the U-118 Mg cells for the 24th hour**
0	100.000
10	95.546
20	88,172
40	61,302
80	2,416
100	3,422

Table 10: The percentage viability of the U-118 Mg cell series in 0, 10, 20, 40, 80 and 100 mg/ml concentrations of milk powder solutions for the 24th hour.



Graph 2: Graph of the comparison of the percentage viability of U-118 Mg cell series in 0, 10, 20, 40, 80 and 100 mg/ml concentrations of milk powder solutions for the 24th hour. The absorbance values recorded by MTT assay kit are used and the percentage calculations are done according to the average absorbance values for each concentration.

Evaluation:

The purpose of this research was to investigate the cytotoxic effect of milk powder on U-118 MG Human Glioblastoma Cell Series. In the processing of the study, different concentrations of the milk powder solution are used. The hypothesis was that the viability of the human glioblastoma cells would decrease when the concentrations of the milk powder solutions are increased. So it means that the viability of the cells in control group in which no milk powder is added would be higher than the trials in certain amount of milk powder is added.

In the experiment, it has seen that milk powder has a cytotoxic effect on U-118 MG Human Glioblastoma Cell Series and there is a significant mean difference between the control group and milk powder added trials. Mean absorbance values recorded by MTT assay was 2.2270 OD for control group, 2.1278 OD for 10 mg/ml of solution added trials, 1.9636 OD for 20 mg/ml of solution added trials, 1.3652 OD for 40 mg/ml of solution added trials, 0.0538 OD for 80 mg/ml of solution added trials, 0.0762 OD for 100 mg/ml of solution added trials.

Standard deviation is smallest in the group in which the concentration of milk powder solution is 80 mg/ml and it is 0.010. So, it can be said that the measurements for the group in which 80 mg/ml of solution is added is much reliable than others.

The null hypothesis of the t-Test was that there wouldn’t be a significant decrease in the viability of the U-118 MG Human Glioblastoma Cells compared with the control group when different concentrations of milk powder solutions are used. Using the t-Test, p values which compare the means of the control group with the means of a trial in which milk powder is added are calculated. For the trials which are done with 20, 40, 80 and 100 mg/ml

solutions of milk powder, p values are smaller than α=0.05, however for the trials which is done with 10 mg/ml solution of milk powder p value is bigger than α=0.05. These show that the null hypothesis of the experiment is rejected for the trials which are done with 20, 40, 80 and 100 mg/ml solutions and there is significant difference between the viability of the cells compared with the control group. But for the trial which is done with 10 mg/ml solution there isn't a significant change in the viability of the cell compared with the control group.

Although there isn't a significant change in the viability of the human glioblastoma cells when 10 mg/ml of solution of milk powder is added, the hypothesis "the number of human glioblastoma cells will decrease when the concentration of milk powder is increased, so there will be an inverse proportion between the number of human glioblastoma cells and the concentration of the milk powder." is mostly confirmed, because the viability of the human glioblastoma cells decrease as the concentration of the milk powder solutions is increased and there is a significant decrease in the number of the cells for the trials in which 20, 40, 80 and 100 mg/ml of solutions are added, so direct proportion between the cytotoxic effect of milk and the concentration of milk powder solution is supported to be true.

Moreover, when p value which is calculated by Anova is considered, it can be said that there is a significant mean difference between the absorbance values for the solutions of different concentrations of milk powder since p value 1.593×10^{-23} is smaller than 0.05.

In the trials in which milk powder is added there is an evident decrease in the viability of the human glioblastoma cells. It is certain that the component of the milk have cytotoxic effect on the human glioblastoma cells and it can be used during the treatment of cancer.

On the other hand; during the evaluation of the data, I have come across with some problems and limitations related with my experiment and they can affect the results in different ways. These limitations are explained below;

1) There are some problems with the concentration values chosen. Although there is a decrease in the viability of the cells for the trial in which 10 mg/ml of milk powder solution is added, the decrease is too small. So, the concentration in which the significant decrease starts should be identified by making some trials. In addition, although the viability of cells in which 100 mg/ml of milk powder is added should be less than the viability of the cells in which 80 mg/ml of milk powder is added, there is a very small percentage increase in the viability of cells for the concentration of 100 mg/ml compared with the concentration of 80 mg/ml. The experiment should have been continued for the concentrations higher than 100 mg/ml and it should have been seen whether the viability of cells still decrease or the concentrations higher than 100 mg/ml affects the cells negatively and increase the number of the cells.
2) In the experiment one type of milk powder which is bought from the market is used. The milk powder used was produced from dried pasteurised cow milk and it isn't known whether the other types of milk powder will bring out the same results or not. In the next experiments related with this subject, different types of milk powder such as powder of breast milk, goat milk, buffalo milk can be used.
3) In this experiment, it is supported that milk powder has a cytotoxic effect on the human glioblastoma cells but the chemical in the milk that causes a cytotoxic effect wasn't examined. For the further works, the chemical ingredients in the milk powder should be investigated. Trials should be done by using each

substance separately and the one which causes the cytotoxic effect should be found out.

4) The cytotoxic effect of milk powder is only studied on U-118 MG human glioblastoma cells. So there is lack of information whether the milk will affect the other types of cancer cells in the same way or not. To overcome with this problem, the experiments with other types of cancer cells such as colon, lung, breast cancer cells, etc, should be done. Thus, the hypothesis can be generalized for many types of cancer cells.
5) In this study, the effect of milk powder is only observed on the cancer cells. However, the effect of milk powder on the healthy brain cells isn't investigated. So, from this study it can be concluded that it is helpful to consume milk for the people who deals with glioma, but it is unknown whether healthy people can consume it. In the further studies, the effect of milk powder on healthy brain cells should be examined. Moreover, this experiment is done in in-vitro conditions due to the limited sources as a high school student. So, the effect of milk powder on glioma cells on a living organism can't be observed. In the next studies the experiment can be done on a living organism by a scientific researcher.

Conclusion:

My research question for the extended essay was “Does milk powder cause a cytotoxic effect on U-118 MG Human Glioblastoma Cells; does the cytotoxic effect that milk powder causes on human glioblastoma cells increase as the concentration of the milk powder solution increases?” The question is answered by preparing a proper experiment method and making statistical analyses with the collected datas. As a result of Anova Statistics, the difference between the number of the human glioblastoma cells that are given different concentrations of milk powder solution is significant and it is shown that the viability of cells decrease with the increasing concentration of milk powder.

The reason for choosing this topic for the extended essay was the confusion in people’s mind about the effect of milk on the cancer patient’s health. Milk is known as one of the most beneficial natural products. However, some scientists state that milk increases the number of cancer cells in a cell line. I made an experiment to clarify the information about the effects of milk on cancer cells, and explained the effect as a high school student. In the extent of limited sources I have, I couldn’t find studies related with the effects of milk on glioblastoma cells, so this essay can be the first study which investigates the effect of milk on glioblastoma cells.

Appendices:

Appendix A:

MTT ASSAY:

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means.[25]

The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.[26]

[25] http://www.atcc.org/~/media/DA5285A1F52C414E864C966FD78C9A79.ashx
[26] http://www.atcc.org/~/media/DA5285A1F52C414E864C966FD78C9A79.ashx

Appendix B:

Neubauer Counting Chamber:

STEP 1. Sample preparation.

Depending on the type of sample, a preparation of a dilution with a suitable

concentration should be prepared for cell counting. Typically, the concentration range for a cell count with Neubauer chamber is between 250.000 cells / ml and 2,5 million cells / ml.

STEP 2. Introducing the sample into the Neubauer chamber

Take 10 µl of dilution prepare in STEP 1 with the micropipette.

STEP 3. Microscope set up and focus.

1. Place the Neubauer chamber on the microscope stage. If the microscope has a fixing clamp, fix the Neubauer chamber.

2. Turn on the microscope light.

3. Start counting the cells in the first square.

4. Write down the amount of cells counted in the first square.

5. Repeat the process for the remaining squares, writing down the counting results from all of them. The higher the number of cells counted, the higher the accuracy of the measurement.

STEP 4: Concentration calculation

We apply the formula for the calculation of the concentration

Concentration (cell/ml) = Number of cells / Volume in mL

The number of cells will be the sum of all the counted cells in all squares counted.

The volume will be the total volume of all the squares counted.

Since the volume of 1 big square is:

0,1 cm x 0,1 cm = 0,01 cm2 of area counted.

Since the depth of the chamber is 0,1mm

0,1 mm = 0,01 cm

0,01 cm2*0,01 cm = 0,0001 cm2 = 0,0001ml = 0,1 µl

So, for the Neubauer chamber, the formula used when counting in the big squares.

Concentration = Number of cells x 10.000 / Number of squares

In case a dilution was applied, the concentration obtained should be converted to the original concentration before the dilution.

In this case, the concentration should be divided by the dilution applied.[27]

[27] http://futurescienceleaders.org/protocols/files/2013/02/Cell-counting-Neubauer-chamber.pdf

Bibliography:

- Elif Deniz Oğuz, “Investigating the apoptotic effect of the diluted nettle (Urtica dioica) juice on rat leukemia cells (Baf3p210 cell series)”
- "Cancer." *Wikipedia*. Wikimedia Foundation, 18 Jan. 2014. Web. 23 Jan. 2014. <http://en.wikipedia.org/wiki/Cancer>.
- "Management of Cancer." *Wikipedia*. Wikimedia Foundation, 20 Jan. 2014. Web. 23 Jan. 2014. <http://en.wikipedia.org/wiki/Management_of_cancer>.
- "National Cancer Institute." *Common Cancer Types* -. N.p., n.d. Web. 23 Jan. 2014. <http://www.cancer.gov/cancertopics/types/commoncancers>.
- "Brain Tumour Foundation of Canada." *: Glioblastoma Multiforme*. N.p., n.d. Web. 23 Jan. 2014. <http://www.braintumour.ca/4869/glioblastoma-multiforme>.
- "Food for Breast Cancer." *FoodForBreastCancer RSS*. N.p., n.d. Web. 23 Jan. 2014. <http://foodforbreastcancer.com/foods/milk>.
- "American Milk: Colon and Breast Cancer Risks." *American Milk: Colon and Breast Cancer Risks*. N.p., n.d. Web. 23 Jan. 2014. <http://www.preventcancer.com/avoidable/colon_cancer/milk_colon.htm>.
- Kyritsis, Athanassios P., Melissa L. Bondy, and Victor A. Levin. "Modulation of glioma risk and progression by dietary nutrients and antiinflammatory agents." *Nutrition and cancer* 63.2 (2011): 174-184.
- "Cytotoxicity." *Wikipedia*. Wikimedia Foundation, 12 Oct. 2013. Web. 23 Jan. 2014. <http://en.wikipedia.org/wiki/Cytotoxicity>.
- "Result Filters." *National Center for Biotechnology Information*. U.S. National Library of Medicine, n.d. Web. 23 Jan. 2014. <http://www.ncbi.nlm.nih.gov/pubmed/22654482>.
- http://www.atcc.org/~/media/DA5285A1F52C414E864C966FD78C9A79.ashx

- "Protocol : Passaging of Adherent Cell Culture Using Trypsin-EDTA." *Bio Infomation Web Index*. N.p., n.d. Web. 23 Jan. 2014. <http://bioinfoweb.com/Protocol-passaging-of-adherent-cell-culture-using-trypsin-EDTA.htm>.
- http://futurescienceleaders.org/protocols/files/2013/02/Cell-counting-Neubauer-chamber.pdf