TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Extended Essay

Research Title: Mutagenic and toxic effects of different concentrations of *Saccharomyces cerevisiae* D7 cells.

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ABSTRACT:

Tobacco use is a very common act among people nowadays. Even though it is known that constant use of tobacco leads to cancer and such serious diseases, there is not quantitative data obtained through experimentation to prove it. However, some researches show that nicotine use can cause several mutations in living organism cells. So, in this experiment both mutagenic and toxic effects of nicotine on living cells are measured. *Saccharomyces cerevisiae* is used as the test organism, since it has the closest structure to human cells. Since mutagenic effects are measured, D7 cells of *S.cerevisiae* that are specially designed by Zimmermann to observe mutagenic effect of a mutagen are used.

First, a proper growth media for *S.cerevisae* cells were prepared and then the D7 cells were put in these cells. In these growth media, nicotine solutions with different concentrations were put, while simultaneously decreasing the concentration of the YEPD solution to maintain the total amount of media in petri dishes. After keeping the cells in these media at $28^{o}C$ for 2 hours, 50 µl of the cells and nicotine- YEPD media mixture is plated onto YPD agar plates then incubated for 7 days at $28^{o}C$ to observe red and white colonies. So, by counting them, quantitative data was gained.

After processing the raw data collected, the results showed that, as the concentration of nicotine increases in the growth media of a cell culture, the number of colonies (living cells) decreases, while the number of red cell colonies increases. Even though this could be said by just considering the raw data, hypothesis needed to be proved in a mathematical way, so Anova: Single Factor was applied to the data. Result of this test showed that the p-values of both toxicity and mutation measurements were smaller than 0,05, so alternative hypothesis were accepted for both of them. This means, at the end of the experiment the toxic and mutagenic effects of tobacco on living cells were both mathematically and visually shown.

I. INTROUDCTION/ BACKGROUND

Yeasts are single-celled fungus that tend to reproduce by budding. Budding is a means of fungal reproduction in which daughter cells are produced as outgrowths of parental cells. *Saccharomyces cerevisiae* is the most important yeast by far in making bread rise and in making wine and beer ferment. As is all this weren't enough, it is a mainstay of scientific research: an entire field of genetics has been built around the study of it.[1]

S. cerevisiae is a very important genetic tool. Since it is very small and unicellular, large numbers of the yeast can be grown in culture in a very small amount of space, in much the same way that bacteria can be grown. However, yeast has the advantage of being a eukaryotic organism, so the results of genetic studies with yeast are more easily applicable to human genetics.[2]

The term mutation is usually reserved for instances in which an allele is known to have been newly formed, such as after treatment of an experimental organism with a mutagen, an agent that causes a heritable change in the DNA sequence.[3]

The standard cell-based assay for mutagenicity testing is the Ames test (Maron and Ames, 1983). This assay is very powerful but cumbersome. There are many cell culture-based assays that can be used to test for mutagenesis, as an alternative or addition to the Ames test. One of them is the method that uses the budding yeast *S. cerevisiae* to test the mutagenicity of compounds (Zimmermann *et al.,* 1975).

This assay use the D7 strain of *S.cerevisiae,* initially constructed and described by Zimmermann *et al.* (1975). When compounds mutate the DNA of this diploid yeast strain, easily scorable phenotypes are produces at three separate genomic sites. Additionally, each one of these phenotypic changes specifies a separate type of mutation and repair mechanism. At the heteroallelic *ade2* site, the two mutant alleles demonstrate intergenic complementation, producing white colonies of yeast. This mutagenesis results in inactive homoallelis ade2 locus. This mutagen-induced ade2 locus can be visualized as red or pink colonies on nutrient-rich media. The heteroallelic *trp5*locus results in tryptophan auxotrophy, and thus D7 cells are

[1] KROGH, David. *BIOLOGY: A GUIDE TO THE NATURAL WORLD.* San Francisco, USA: Pearson Education, Fourth Edition.

[2] Volk, Tom, Galbraith, Anne. December, 2002. 10:30 pm, 30 December 2010. Saccharomyces Cerevisiae: the beakers' and brewers' yeast. http://botit.botany.wisc.edu/toms_fungi/dec2002.html

[3] Lodish, Harvey; Berk, Arnold; Kaiser, Chris A.; Krieger, Monty; Scott, Matthew p.; Bretscher, Anthony; Ploegh, Hidde; Matsudaira, Paul. *MOLECULAR CELL BIOLOGY.* New York: W. H. Freeman and Company, Sixth Edition.

not able to grow on synthetic media lacking tryptophan. Mitotic gene conversion at the *trp5* locus, caused by repair mechanisms induced after particular types of mutagenic treatments, produces colonies that are able to grow on synthetic media lacking tryptophan. Furthermore, reverse mutations can be identified by assaying for isoleucine prototrophy. The D7 strain is auxotrophic for isoleucine because of its homoallelic loss-of-function mutations in the *ilv1* locus. This isoleucine auxotrophy can be overcome by a reverse point mutation at the *ilv1* locus. To summarize, in the laboratory setting, the *S. cerevisiae* tester strain D7 can be treated with a compound that is a potential mutagen and phenotypic changes can be followed to indicate mutagenesis. Depending on the type of phenotypic change, the activity of the mutagen can then be inferred.[4]

The Nicotine is a very common and harmful matter. One can say that it is used in many ways and very often, such as in cigarettes, tobacco etc. Even though tobacco contains many other poisonous ingredients, it can be said that nicotine is one of the most dangerous and addictive one of them. As I observe the use of tobacco very frequently in the environment I live, I have decided to design an experiment to understand and show its harmful effects on a living organism better. Also, after a research process, I have found out that the relation between cancer and such disease and nicotine intake is not quite proven via experimentation and quantitative data. So, in my experiment I have decided to measure the mutagenic and toxic effects of nicotine so that it might help one to relate the diseases like cancer with nicotine use.

Main issue that is handled in this research is the effect of nicotine on living organisms. To make this research, the issue is specified by choosing a certain type of tobacco to get different concentrations of nicotine and one living organism to get focused results, yeast cells (D7 cells of *S. cerevisiae*).

My research question is: *How does the change in concentration of nicotine solution in the growth media influences the mutagenic and toxic effects of nicotine on Saccharomyces cerevisiae D7 cells?*

[4]Marshall, Pamela A.. 2007 Winter. Time not stated. 11:09 pm, 25 October 2011.
Using *Saccharomyces cerevisiae* to Test the Mutagenicity of Household Compounds: An Open Ended Hypothesis-Driven Teaching Lab.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2104512/

II. HYPOTHESIS

Tobacco smoking is the practice where tobacco is burned and the vapors either tasted or inhaled[5].

Tobacco use leads to diseases that most commonly affects the heart and lungs, making smoking a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD), emphysema, and cancer (particularly lung cancer, cancers of the larynx and mouth, and pancreatic cancer)[6].

According to the information above, the cigarettes include various types of harmful ingredients along with tobacco, but nicotine is one of the most dangerous one of them since it can also be used separately from cigarettes. So, it was hypothesized that there will be a significant difference between the toxic and mutagenic effects of different concentrations of nicotine that is extracted from a certain tobacco type on D7 *Saccharomyces cerevisiae* . It is expected that as the concentration of nicotine in the medium of a cell increases, more toxic and mutatic effects will be observed. So due to this alternative hypothesis, my null hypothesis is that there will be no significant difference in the cell number and the number of mutated red cells as the concentration of the nicotine in the growth media changes.

It should be stated that this study only invastigates the effects of nicotine on the *Saccharomyces cerevisiae*. So, the results of this investigation are not enough to make a generalization about the effects of nicotine on humans. So it can't be said that nicotine is proven to cause mutation in human cells by only taking the results of this study.

III. METHOD DEVELOPMENT AND PLANNING

As early as the 1600s, people speculated that there might be a link between diseases, like cancer, and tobacco use. Since then, modern research methods have provided evidence of this link, and public service announcements that warn of tobacco's health risks and addictive nature are seen regularly on TV. Despite those warnings many people still continue smoking. There are several studies that show the relationship between smoking and getting certain types of cancers and also early aging. Those studies mostly focused on the relationship between

undergone cancer types and smoking habits of the patients. I wanted to show the direct relationship between smoking and getting cancer and cell aging as there is not such a direct scientific study published yet. Also I wanted to show the toxic and mutagenic effects of smoking evidently. First I searched the hazards of smoking and where the hazards mostly come from; the nicotine itself or the additives? After some literature survey I found that both parts have dangerous effects on human health. But it was difficult for me to test the toxic effects of ingredients inside the cigarettes because in liquid media they might have lost their characteristics or those substances might not get into growth media of cells which I am going to choose. So I decided to test the mutagenic and toxic effects of nicotine. I searched for pure nicotine. But it was very expensive and also dangerous to handle. So I extracted my own nicotine which was not so much concentrated but enough to reach the aim of my experiment. I used water extraction method. As nicotine is soluble in water this method worked very well. Then I had to choose an organism which shares similar characteristics with human cells. I decided to use *S. cerevisiae* cells in my studies as this organism is a eukaryotic one. *S.cerevisiae* cells are also easy to handle without any pathogenic characteristics. I could test the toxic effects of nicotine on S.cerevisiae cells but testing the mutagenic effect of nicotine on those cells was very difficult for me as I have little molecular biology background. Cells which I was going to choose also had to exhibit mutagenic effects that may lead to cancer. After literature survey, I found *S.cerevisiae* D7 cells which were designed by Zimmerman et. al. in 1970. By using those cells I could test both toxic and mutagenic effects of nicotine. My supervisor asked Dr. Pamela Marshall from Arizona State University whether she could send those cells. Dr. Marshall kindly sent those cells to us for our work. Then I have learned aseptic microbiological methods in the Laboratory of Prof. Dr. Fatih Izgü at Middle East Tech.University. After extraction of nicotine, I decided to use five different concentrations of nicotine. For the testing of toxic effects of nicotine on S. cerevisiae cells I could choose two methods. One of the methods was measuring the absorbance of cells treated with different concentrations of nicotine at 480 nm spectrophotometrically. Although this method is easy to perform and not time consuming, I did not choose this method. Because it only shows the concentration of cells, not exactly the number of living cells. In other word it does not differentiate the living and dead cells. So I decided to use colony counting method. Although this method is time consuming and requires massive working like preparing petri plates in large quantities, it shows exact number of living cells providing what I exactly needed to test the toxic effects of nicotine. Also by choosing this method I could count the number of colonies which are mutated. For the counting of colonies, I diluted the cells which I had

treated with nicotine and also the control group without nicotine in order to prevent forming of massive uncountable colonies. I was very careful when performing aseptic microbiological techniques in order to prevent any cross contamination that would interfere with the results of the experiments. During all microbiological experiments and extraction of nicotine, I used dry air or ethylene oxide to sterilize laboratory equipments. In order not to influence the composition of growth media, I used double distilled steam sterilized water throughout the experiments. To spread the cells which were incubated with different concentrations of nicotine onto YEPD agar plates, I used high quality glass rod from Sigma Company. I burned the glass rod each time before inoculations with ethanol and waited some time to cool the glass rod not to damage the cells by excess heat. In order to maintain the volume of cells, I have used adjustable accurate micro pipettes with sterile tips. After putting the adjusted volume of cells onto YEPD plates, I turned the plate two times while spreading with glass rod in order to accurately spread the cells. After spreading the cells I put the petri plates into an incubator at 28^0C and incubated them for 3 days. After three days I counted the colonies formed on the YEPD plates using small tipped board marker. I labeled the colonies which I counted with the tip of a board marker not to count the colonies two times.

A. Materials used in the experiment:

1. *S. cerevisiae D7 Mata/Matα ade2-40/ade2-119 ilv1-92/ilv1-92 trp5–12/trp5-27* cells.
2. Virginia nicotine (5 g)
3. An autoclave with the temperature set to 121^o and a pressure of 1 kg per cm^2 above ambient pressure (autoclave 15 min). (ALP –Japan)
4. An oven for temperatures of 160^0 to sterilize glassware and other dry goos (3 h at 160^0) (Heraus, Germany)
5. A water-bath to cool solid media to 45^0 after they are autoclaved and before they are poured into plates.
6. A roller drum or a reciprocal shaker for growing cultures in test tubes in liquid medium.
7. Vortex mixer to mix liquids in test tubes.
8. A pH meter to adjust the buffers.
9. An analytical balance for weighing mg portions.
10. Beakers 10x100 ml, 10x500 ml, 10x 1000 ml.
11. Pipettes (Gilson, France), all with blow-out calibration.
12. Sterile plastic petri dishes with inner diameter of 90-100 mm. (Interlab, Turkey)

IV. METHOD

A. Yeast Strains:

S. cerevisiae strain D7 Mata/Matα ade2-40/ade2-119 ilv1-92/ilv1-92 trp5–12/trp5-27 was generously provided by Dr. Pamela Marshall (Arizona State University, USA).

B. Isolation of Nicotine from tobacco:

1. 5g Virginia nicotine dissolved in 100 mL of dd H_2O and homogenized by using a hand blender (Braun, Germany)
2. The solution is incubated via shaking at 150 rpm (Innova 4500, New Brunswick, USA) overnight at 37^{o}C.
3. After overnight incubation the tobacco solution is put into 50 mL centrifuge tubes and they are centrifuged at 3000 rpm at 10^{o}C in order to get rid of tobacco particles. (Centrifuge; BR4i, Jouan, France) (see: Appendix 2)
4. The supernatant which contains nicotine, then filtered through 0.45 μm filter (Sartarius, Germany) to sterilize the solution. This solution is kept at 4^{o}C until use.

C. Preparation of Yeast Extract, Peptone, Dextrose (YEPD) media:

1. YEP medium consists of 1% yeast extract, 2% peptone (BD Bioscience, USA) supplemented with 2% glucose (Merek, Germany) as the usual carbon source and pH of the growth media is adjusted to 6 with hydrochloric acid (Sigma, USA).
2. For solid media 1.5% of agar (BD Bioscience, USA) is added and the mixture is autoclaved at 120^{o}C under pressure with everything together (ALP, Japan).
3. After autoclave, agar media has to be thoroughly mixed, cooled to 45^{0}C and then poured into petri dishes under sterile conditions. (see: Appendix 2)
4. After incubating the poured plates for two days at room temperature, they are ready to be used.

D. Isolation of non-revertant D7 cells:

The D7 strain was plated directly form the frozen stock onto YPD plates several weeks before the scheduled lab date. This plate was incubated at 28^{o}C for 3-4 days. Once colonies had formed, the color of the colonies was checked to confirm the vast majority of colonies were white (greater then 95%). Five to ten white colonies were then individually picked and grown overnight in 10mL YPD media at 28^{o}C with shaking at 150 rpm. The following day aliquots of each individual YPD liquid culture were plated onto YPD plates. The plates were incubated at 28^{o}C for upto a week and phenotypes were checked. Once a liquid culture had been identified that had the least amount of revertants, this liquid culture was used as a starting culture

(1ml) for an overnight culture in 10ml YPD, grpwn at 28^{o}C with shaking (150 rpm). The cells were centrifuged, the medium was removed and the cells were resuspended and diluted 100 times in fresh YEP media (pH 6.0).

E. Treatment of D7 cells with nicotine:

-treatment conditions:

1. Treatments should be carried under constant temperature and shaking. A temperature-controlled water-bath at 25^{o}C (Nüve, Turkey) is used.

2. Treatments should be performed in buffered solutions. The strength of the buffer has to be adjusted so as to compensate possible influences of the dissolved chemical on the overall pH.

-treatment of cells with nicotine:

Resuspended and 100 times diluted *S.cerevisiae* D7 cells were treated with different amounts of nicotine solution. For this, 100 µl of diluted *S.cerevisiae* D7 cells were inoculated in 10 µl Falcon tubes (BD Bioscience, USA). The control tube (without nicotine) contained only 4900 µl YEP media at pH 6.0 and the cells. Then to each tube, required volumes of nicotine solutions (0 µl, 200 µl, 400 µl, 600 µl, 800 µl) were added along with the cells. Then, to each tube necessary volume of YEPD medium (pH 6.0) was added to reach to the total volume of 5000 ml. Tubes were incubated for two hours .

After incubation, 50 ml of the cells and the nicotine - YEPD media mixture were directly plated onto YPD agar plates (pH 6.0) via glass nod. Then, they were incubated for 7 days at 28^{o}C.

After incubation, the total colonies formed on each plate was counted. Also, the mutated red colonies formed were counted and recorded.

For each nicotine solution concentration, 5 trials were prepared in the same way.

V. DATA COLLECTION AND ANALYSIS:

Volume of Nicotine Solution (±5 µl)	trials	Total number of colonies	Number of red colonies with mutations	Controlled Variables			
				pH of the growth media and YPD	Temperature of the incubation environment (^{o}C)	Incubation time of cells (days)	Volume of cells inoculated to growth media (µl)
	1	1421	21	6.0	28	7	100
	2	1413	23	6.0	28	7	100
	3	1426	24	6.0	28	7	100
	4	1414	19	6.0	28	7	100
0 µl	5	1425	20	6.0	28	7	100
	1	974	68	6.0	28	7	100
	2	975	69	6.0	28	7	100
	3	956	66	6.0	28	7	100
	4	962	68	6.0	28	7	100
200 µl	5	972	70	6.0	28	7	100
	1	887	85	6.0	28	7	100
	2	878	80	6.0	28	7	100
	3	866	78	6.0	28	7	100
	4	881	76	6.0	28	7	100
400 µl	5	874	82	6.0	28	7	100
	1	794	92	6.0	28	7	100
	2	784	88	6.0	28	7	100
	3	776	86	6.0	28	7	100
	4	764	85	6.0	28	7	100
600 µl	5	798	93	6.0	28	7	100
	1	502	110	6.0	28	7	100
	2	492	105	6.0	28	7	100
	3	530	120	6.0	28	7	100
	4	510	108	6.0	28	7	100
800 µl	5	508	110	6.0	28	7	100

Table1: In this table, the raw data obtained after the treatment of D7 cells with different concentraitons of nicotine solutions in shown. The number of white and red colonies were recorded for 5 trials of 5 different concentrations.

Statistical Analysis for the cell number change in the colony:

	0	*200*	*400*	*600*	*800*
Mean	1419,8	967,8	877,2	783,2	508,4
Standard Error	2,709243	3,746999	3,512834	6,151423	6,241795
Median	1421	972	878	784	508
Mod	#NONE	#NONE	#NONE	#NONE	#NONE
Standard Deviation	6,058052	8,378544	7,854935	13,755	13,95708
Variance	36,7	70,2	61,7	189,2	194,8
Kurtosis	-2,9136	-1,65985	0,298328	-1,01028	1,608853
Skewness	-0,24153	-0,81643	-0,37924	-0,47217	0,828883
Interval Estimation	13	19	21	34	38
Greatest	1413	956	866	764	492
Smallest	1426	975	887	798	530
Sum	7099	4839	4386	3916	2542
Count	5	5	5	5	5
Confidence Interval(95.0%)	7,522066	10,40334	9,75319	17,07909	17,33

Table2: In this table, the statistical analysis resutls for the change in the cell number of a colony due to different concentrations of nicotine is summarized.

Anova: Single Factor

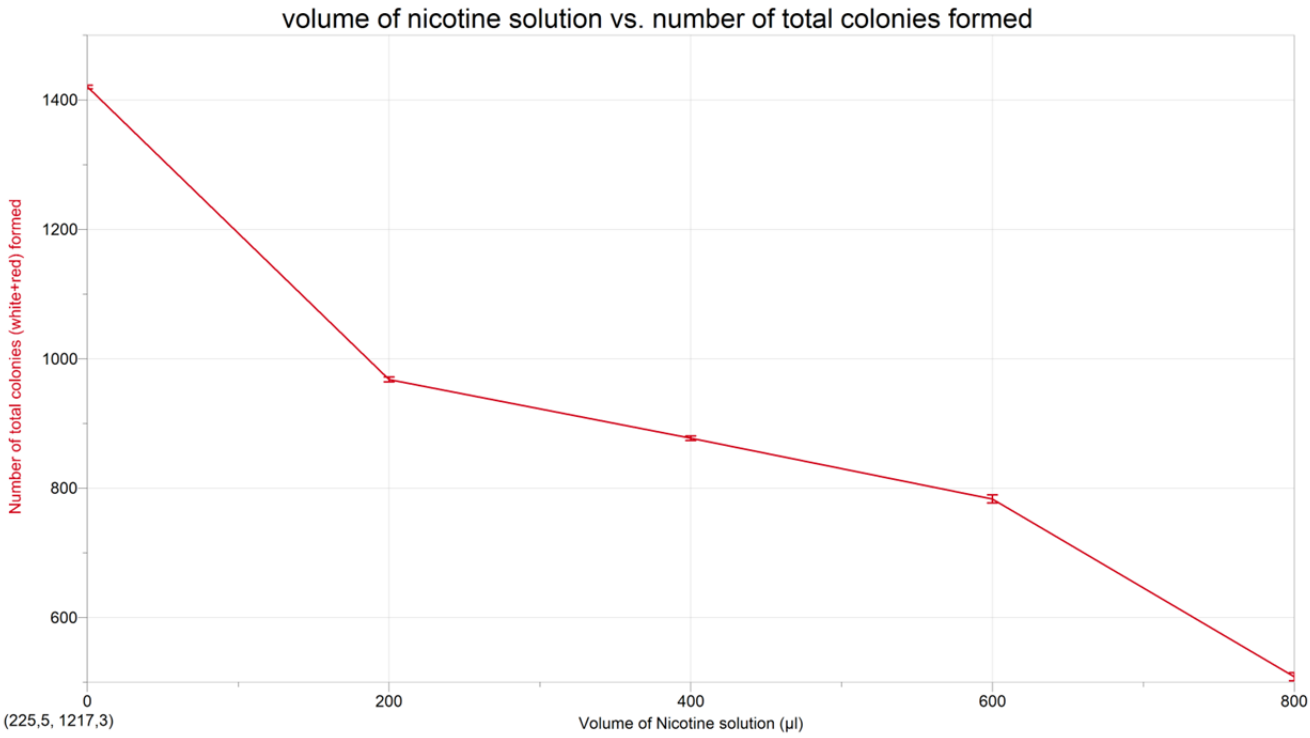
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	5	7099	1419,8	36,7
200	5	4839	967,8	70,2
400	5	4386	877,2	61,7
600	5	3916	783,2	189,2
800	5	2542	508,4	194,8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2208327	4	552081,7	4995,31	1,1E-29	2,866081
Within Groups	2210,4	20	110,52			
Total	2210537	24				

Table3: In this table, the Anova: Single Factor results of the data that shows the change in the cell number of colonies due to a change in the concentration of nicotine in the medium is shown.



Graph1: In this graph, the relationship between the concentration of nicotine in the medium of the cells and number of the living colonies in that media is shown.

Statistical Analysis for the red cell number change in the colonies:

	0	*200*	*400*	*600*	*800*
Mean	21,4	68,2	80,2	88,8	110,6
Standard Error	0,927362	0,663325	1,56205	1,593738	2,521904
Median	21	68	80	88	110
Mod	#YOK	68	#YOK	#YOK	110
Standard Deviation	2,073644	1,48324	3,49285	3,563706	5,639149
Variance	4,3	2,2	12,2	12,7	31,8
Kurtosis	-1,96322	0,867769	-0,64364	-2,68027	2,923342
Skewness	0,235514	-0,55162	0,309766	0,271769	1,479995
Interval Estimation	5	4	9	8	15
Greatest	19	66	76	85	105
Smallest	24	70	85	93	120
Sum	107	341	401	444	553
Count	5	5	5	5	5
Confidence Interval(95,0%)	2,574769	1,841685	4,336946	4,424925	7,001928

Table4: In this table, results of the statistical analysis for the change in the red cell number of a colony due to different concentraiton of nicotine is summarized.

Anova: Single Factor

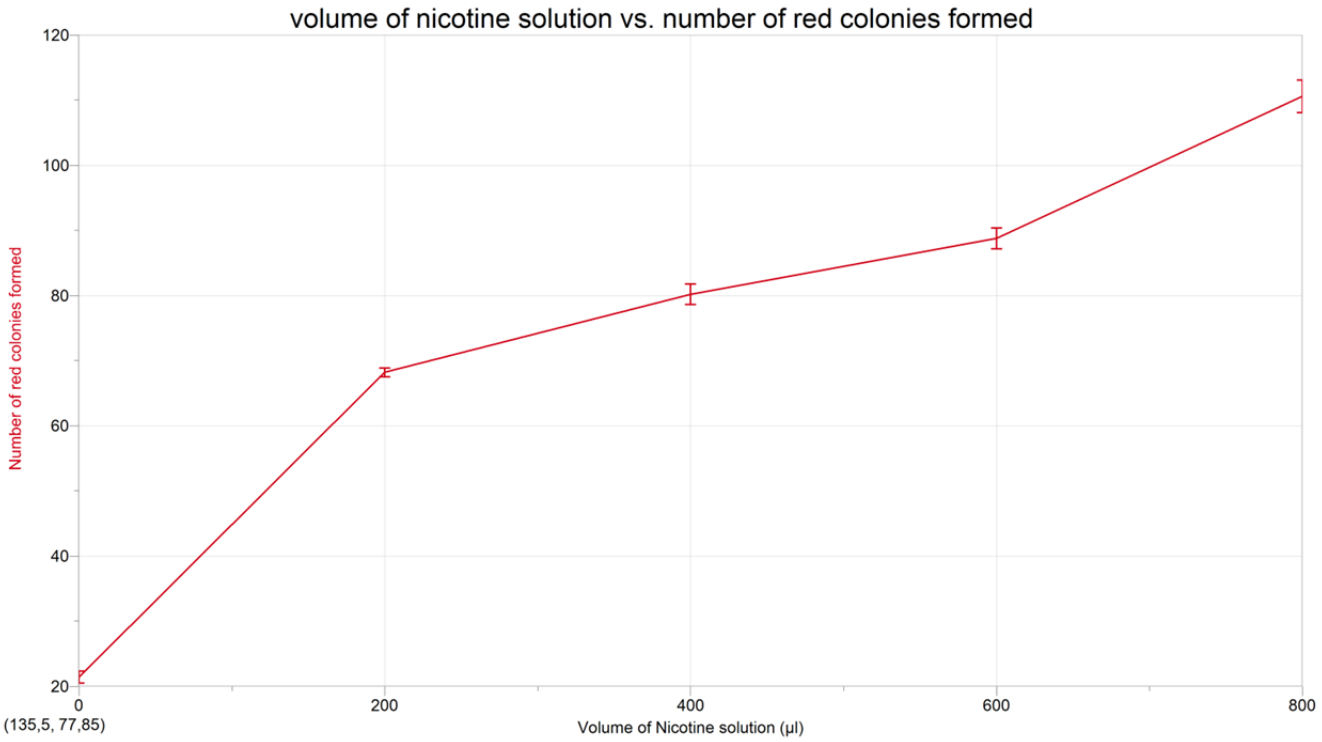
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	5	107	21,4	4,3
200	5	341	68,2	2,2
400	5	401	80,2	12,2
600	5	444	88,8	12,7
800	5	553	110,6	31,8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21986,56	4	5496,64	434,8608	3,92E-19	2,866081
Within Groups	252,8	20	12,64			
Total	22239,36	24				

Table5: In this table, the Anove: Single Factor results of the data that shows the change in the red cell number of colonies due to a change in the concentration of nicotine in the medium is shown.



Graph2: In this graph, the relationship between the number of mutated red colonies and the nicotine concentration in the medium of the cells is shown.

The experimental method that I designed gave satisfactory results. I could easily observe and conclude the toxic (lethal) and mutagenic effects of nicotine. So, I observed that as the nicotine concentration increased, the toxic and mutagenic efects on the cell colonies also increased while reducing the number of white cells and increasing the number of red cells. The statistical analysis of the raw data that were collected also proved these qualitative results.

After applying Anova: Single Factor test on my data, I saw that the P-values were smaller (for number of cells: $1.1*10^{-29}$, for number of red cells: $3.92*10^{-19}$) than the α-value, which is 0.05. As a result, I had to reject my null hypothesis and accept the alternative hypothesis for both measurements; toxic effects and mutagenic effects.

H_0: Nicotine concentration does not have a significant affect on the total numer of cells and the number of red-mutated cells in a colony.

H_A: As the concentration of nicotine in the medium of *S. cerevisiae* D7 cell colony increases, the total number of cells in the colony will decrease, whereas the number of mutated red cells will increase.

Standard Deviation shows how much a set of data is disseminated due to mean value. So if SD is minor, it means there is a small amount of inconsistency between the collected data, thus less errors in the experiment. From tables 2 and 4, it is seen that for both red colonies and total number of colonies, SD values are not very small, not under 6,0. Also it is seen that, as the nicotine volume in the growth media increases, the SD values increase. These values' not being small shows that there are some points that the method or measurements of this expeirment come short. In addition, it is seen that the SD values of red colonies and total colonies are very close to each other. This may be the proof of how stable the experiment environment was kept.

Also, in graph1, the relation between the concentration of nicotine and the number of living cells after 2 hour incubation with different volumes of nicotine solution can be seen. It seen that as the concentration of nicotine extract increases, the number of colonies formed decreases. In graph2, the relation between the number of mutant cells, that are red, and the concentration of nicotine exract in the medium is shown. It is seen from the graph2 that as the concentration of nicotine extract in the medium increases, the number of mutant red cells increases as a proof of the nicotine's effect on the adenine gene mutation.

VI. LIMITATIONS AND EVALUATION:

I have tried to perform the experiment with extreme caution to be able to get reliable results. I have used 70% ethanol solution for sterilization of all the equipments that is involved with the experiment. Also, I used specially designed glass stirrers to disseminate the cells on the petri dishes to prevent the cell from sticking on the stirrer.

The most difficult part of my research was preparing sterile petri dishes with YEPD media inside and counting the colonies. YEPD was poured into the petri dishes while it was warm and petri dishes were kept closed till the last moment to make it more sterile. Also, I have counted each petri dish twice to prevent any miscount, since this type of a mistake might have caused a great error in the results. Even though I tried not to make any mistakes and took cautions, at the end I thought I could design a better experiment.

First of all, I could have tested other types of mutations, such as reverse mutation, but that required a specific growth media. Though I searched for that type of media, I was not able to purchase them due to their cost. So, this probably caused my research to have a smaller area of validity.

Also, I think that it would be better to test mutagenic and toxic effects with animal cell culture, since it has a closer structure to that of human cell. I contacted some laboratories that are experienced in that field, however they told me that I needed to get experienced for at least five months to perform this type of experience. So, I continued my research with special type of s. cerevisiae D7 cells, which were just appropriate to get convincing results. However, not being able to perform my research on cells that are closer to that of humans' might have caused its result to be less applicable to all type of human cells, thus have a smaller range.

Another important change that I could have done to make my experiment better is the dilution factor of the seeded colonies. While I was counting the colonies, I realized that some colonies were attached to each other and it was difficult to count them with full accuracy. So, it would provide more accurate data and make it easier to apply the counting process if I had diluted the colonies more.

When treating the D7 cells with different volumes of nicotine concentrations, I had to use different volumes of YPD media (decreasing 200 µl each time the volume of nicotine solution increases 200 µl). This changed the total composition of YPD media in each tube. So there was 800 µl of decrease in the growth media with 800 µl of nicotine solution. However, through my research, I discovered that the composition of YPD media has a negligable effect on the growth of yeast cells. In fact, I found out even when the media contents are reduced by 50%, growth of yeast cells is not effected. Though, I could eliminate this small effect if I had solid nicotine.

One weakness of the experiment was the measurement of the concentration of cells. I think it would make it possible to determine the toxic effects of nicotine if I had measured the density of cells also spectrophotometrically. The reason why I considered a spectrophotometre is because it would give more accurate readings than counting with human eye, since it has only ±0.0001 of uncertainty.

By eliminating obsticles like the ones above, errors might be minimized and more accurate results can be obtained.

VII. CONCLUSION:

After all the data collection and analysis, I was able to observe the mutagenic and toxic effects of nicotine by the experimental method that I designed. I prepared about 50 petri dishes and I performed 5 trials of colony forming unit counting for each concentration of nicotine. As a result, it was proven that there was a direct relation between the concentration of nicotine and the number of total cell colonies and red cell colonies, thus the mutagenic and toxic effect of nicotine.

Although the experiment had some significant limitations, it can be considered an important research on the effects of nicotine on eaukaryotic cells, thus human cells.

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2. Author's name not stated. Date and time not stated. 11:23 pm, November 2011.

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IX. APPENDICES:

A. Appendix 1:

In these pictures, the colonies formed, from *S. cerevisiae* D7 cells that were treated with different volumes of nicotine, on YPD media after 7 days of incubation are showed:

Picture1: 800 µl nicotine solution.

Picture2: 600 µl nicotine solution.

Picture3: 400 µl nicotine solution.

Picture4: 200 µl nicotine solution.

Picture5: 0 µl nicotine solution.

Picture6: Starter colonies (with little red revertant cells) that are chosen and used for the testing the effects of different concentrations of nicotine.

B. Appenix 2:

Picture7: I am preparing the nicotine solution by putting them in 50mL centrifuge tubes to be centrifuged.

Picture8: Nicotine solutions and growth media and cells are being mixed via vortex before incubation.

Picture9: Tobacco solutions are being prepared for centrifuge in 50mL centrifuge tubes by me.