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The Mutagenic and Cytotoxic Effects of Exposure to Heavy Metals and other Oxidative Species from E-cigarettes on Epithelial Cells

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By

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Abstract

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The goal of this project was to investigate the effects that the products present in the components of e-cigarettes would have on the cellular level. It was postulated that the presence of heavy metals or other reactive oxidative species (ROS) would cause damage and either mutate the cells or result in cellular death. The experiments that were carried out focused heating element and the vape juice. The experiments regarding the heating element involved treating the media with an electrified heating element then observing the effect on the cells. There were significant limitations with this study so most of the data came from the vape liquid experiment. The vape liquid experiments involved treating the media with an absolute ethanol and specific value of vape liquid present in a low and high concentration test group. The reasoning for the addition of the absolute ethanol will be discussed later, and a test group treated with ethanol was ran to prevent any inaccurate attribution of damage to the wrong substances. The results showcased that there was a mitogenic quality to the vape juice tests with cellular proliferation rates increasing. Although, p53 data was not able to be obtained, it was thought that there would be p53 expression due to potential DNA damage or damage to the cell cycle checkpoints.

Keywords: e-cigarette, cytotoxic, mitogenic, vape, heavy metal, oxidative species

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Introduction

There is an epidemic that is currently raging in the lives of many adults and young adults in our country. This epidemic is the use of e-cigarettes or vapes. These are branded as a safer alternative to traditional cigarettes. However, this may not be the case. Vaping is easier to conceal and allows people to use the products more often and in situations where they could not smoke traditional cigarettes. For example, workers can quickly use the e cigarette, inhale, and keep most of the vapor and have little residual odor or proof of use. Inappropriate use like the full inhalation is different and can result in higher concentrations of molecules encountering the respiratory tract and the rest of the body via the bloodstream. Vapes are also pushed to the limits in order to give the greatest buzz or produce the greatest amount of vapor. These manipulations and quick use can push the mechanisms outside of their normal safety testing ranges and can lead to increased dangers of releasing toxic chemicals. This is especially true for juveniles and young adults who may pursue vaping because of the stigma that it is a cool thing to do, as well as they may chase the buzz. There are thousands of assorted flavors that cater to the sweet fruity flavor that many young individuals would find attractive. This is especially detrimental to health because the bodies of people are not fully matured until around the age of 25. So any damage could have extreme downstream effects. With the use of these electronic dispense methods, the heating elements are often pushed beyond the limits of what is advised by the producers of these products. This can increase the exposure to damaging chemicals. These chemicals include heavy metals such as chromium, manganese, cobalt, nickel, arsenic,

and cadmium. There are also other types of harmful and potentially harmful constituents (HPHC) (Pappas et al. 2014). These are oxidative species which can wreak havoc among intracellular machinery and DNA. Exposure to these chemicals can cause mutations to occur which can cause many issues for cellular growth and development. In some cases exposures can lead to cell death as well as potential cancer formation. Exposure to these chemicals can be especially detrimental to young adults whose body systems, such as respiratory and nervous, are not fully matured until ages 20-25. The goal of this study is to observe the effects of various treatments of vapor on epithelial cells. The hypothesis is that if the cells are treated with these HPHCs, then there will be increased cellular damage and death that occurs in these cultures. These tests will be discussed further in the experimental methods section.

Essential Materials

There are several important items that were used within the experiment, and they will be discussed in detail here. Perhaps the most important piece of materials that were used would be the cells. Chinese Hamster Ovarian cells (CHO) were used in the experiments. These cells were chosen because of their relatively cheap cost and similar behavior to human epithelial cells. For the purposes of this experiment the cellular reaction to the test groups would elicit a similar reaction to how human cells would react to the same environment. This was necessary as working with human tissue is considered unethical by many, as well as requiring extensive Institutional Review Board approval. Not to mention the cost of such cells would vastly surpass the funds allotted for this experiment. The next required item is the cell culture media. Dulbecco's Modified

Eagle Media (DMEM). This media contains many growth factors and important nutrients such as L-glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate. These materials allow the cells to grow and develop in what would be an ideal growth environment. The media also was supplemented with a few other materials to bolster its ability to grow cells. The supplements were fetal bovine serum (FBS) and penicillin/streptomycin. The FBS is used to supplement additional growth factors and nutrients to the media, while also serving as a protectant against large pH shifts, proteases, and toxic elements while the cells grow and develop. The penicillin/streptomycin is an antibiotic used to prevent bacterial contamination within the cultures. The containers that the cells are grown in are an important part of the culture process. The T25 and T75 series flasks were the primary containers used as they have cellular connections present for the cells to adhere to the flask. This allows the cells to return to normal growth and development. These caps often contained vents to allow CO_2 to enter the flasks, although some required the caps to be manually vented. An incubator is required to allow the cells to be grown at 36℃ and with a CO_2 saturation of 6%. It is paramount the cells are kept in these conditions otherwise the cells can leave normal metabolic rates and die. A way to remove the cells from the flasks to run tests or split them is important. A trypsin/EDTA solution was used to separate the cellular connections to the plate, although a manual scraper could be used. A centrifuge with the capabilities to generate 300 g of force for 5 minutes is needed to be able to wash the cells of cold storage medium or of trypsin. Among the most important materials are the pipettes. These come in a variety of forms ranging from 5 mL to 25 mL. These come in sterile packaging and must be opened within the hood. This is important because the

pipettes will touch the sterile inside of the flasks where the cells are growing, and any contamination can have disastrous results.

Experimental Procedure

The experimental analysis of this project will be carried out in the lab. The experimental setup requires the culturing of the cell line. The cell line in the experiments are CHO cells, fibroblast-like. These cell lines are grown in sterile environments with DMEM, supplemented with FBS and treated with a combination of penicillin and streptomycin. Once the cell line has grown, they will be plated and treated with the various concentrations of the vape heating element and/or vape juice.

The first preparation step for the culture project is to prepare a bottle of media. The Dulbecco's modified eagle media that was used came in 500mL bottles. Each bottle was supplemented with 50 mL of penicillin/streptomycin. The penicillin and streptomycin serve to protect cells from bacterial growth that may occur from any contamination into the system. The media also is supplemented with 50 mL or 10% by volume of fetal bovine serum.

Once the media is made the process of culturing the cells out of cold storage is the next step. Cells are usually kept in liquid nitrogen for best preservation but can be moved to the -80°C before the culturing. Once the cells are removed from cold storage, they are placed into a hot water bath at 36°C to thaw. While the cells are thawing it is important to ensure that the hood and everything inside the hood is sterile. Every time one enters the hood, one must wear gloves that have been sprayed with the 70% ethanol solution. It was made a widespread practice to spray a 70% ethanol solution

into the hood and wipe down the surfaces to ensure that nothing was growing. Every item that went into the hood, which was not already sterile, was also wiped down with this solution. The new media and trypsin/EDTA solution was also placed into the hot water bath to incubate to room temperature so as to not shock the cells when they are treated. Once the cells are removed from the bath the process of washing the cells. The cells must be washed because the storage medium outside of the cold is cytotoxic, but it helps to preserve the cells for extended periods in cold. The process of washing the cells begins by removing the cellular solution from the vial and placing it into a 15mL tube. Media is used to get the overall volume of the tube to approximately 10mL. Then the tube with the cells was placed into a bucket centrifuge at 300g for 5 minutes. The cells are present in the pellet at the bottom of the tube once the tube is removed from the centrifuge. The supernatant of the tube is carefully discarded without disturbing the pellet. Then 10 mL of media is again added, and the tube is again centrifuged at 300 g for 5 minutes. Once again carefully discard the media without disturbing the pellet. Now the cells are clean and fresh media can be added to plate the cells. The T25 plate was the primary plate that was used to receive initial cell cultures. One would typically plate 4-5mL of cell solution in each plate and 5-6mL of fresh media, 10 a final volume of 10mL, to each T25. It is paramount when plating the cells to ensure that there is no contamination. This means to be extremely aware of where the tip of your pipette is at all times, as well as not touching any surface that would have contact or could have contact with the inside of the flask. Once the cells are transferred carefully into the flask, the flask is labeled with the cell type, passage number, researcher initials, and date. Passage number refers to the number of times that the cells have been transferred to a

new flask, or split. The cells are monitored every 3 days, and likely need to change the media, or split the cells, depending on the confluency. At approximately 80% confluency the cells need to be split into separate flasks. This means that cells cover around 80% of the visible surface within a microscope view. The cells were cultured until there was an excess of flasks necessary to provide the data that we wished to obtain.

Experiment 1 Setup

Remove the heating element system from a JUUL brand vape. Attach the heating element to a power source (9-volt battery). Treat the media that the cells would be plated in for 1 minute (low concentration) and 2 minutes (high concentration). Then plate the cells into the treated media on a 6 well plate for observation. The first iteration involved solid 18 ga copper wire. The wire was to be attached to the heating element probes, this was accomplished by clamping the wires around the probe with pliers, although there were some limitations with this setup that will be discussed later. Then the entire system was to be autoclaved to ensure sterility. The system was then placed into a 50 mL tube containing media, then the system was attached to the battery to pass the charge into the heating element. However, the probe detached resulting in another round of autoclaving and the development of a new system. The second iteration involved 18-gauge braided wire which proved to be more flexible and easier to work with. The same process was repeated involving the autoclave process and the insertion into the 50 mL tube of media. The treatment groups would have 1 minute of electricity treatment for the low, and 2 minutes of electrical treatment for the high group. There

were also limitations associated with this treatment as well which will be discussed later as well. Thus, the second experiment was created.

Figure 1: The second iteration of this apparatus utilized braided wire and improved the connections to the heating element and the battery. The first iteration can be seen above in the autoclave bag, although the same heating element and battery were used.

Experiment 2 Setup

The second experiment set out to explore the potential of the vape juice to damage the cells or to observe the effects of the treatment on the cells. The first step was to obtain a bottle of vape juice liquid with a concentration of 50 mg Nicotine for 30 mL of liquid (1.67mg/mL). The juice had to be solubilized in absolute ethanol (EtOH) in order to prevent it from separating from the media. The treatment groups were 30 µL

(0.025µg/mL) and 300 µL (0.25µg/mL) in 20 mL of absolute ethanol. Then plate the cells into the media on a 6 well plate and treat with 50 µL of the corresponding test.

General Limitations

The fundamental issues that were encountered within the lab, contrary to what was preliminary expected, was unexpected cellular death. The cells would appear to be doing well and the cells would be split to allow for further growth and development but then the cells would not attach and die. The first few rounds we struggled to determine what the reason was behind the cell death. It was postulated that perhaps there was an issue with the trypsin/EDTA solution that we were using to remove the cells from the plates. It was thought that the trypsin was becoming residual or destroying cellular machinery to the point where the cells could no longer attach. We discarded the old trypsin and used new assays and we thought perhaps this would solve the issues. However, the cells continued to die soon after plating. The next step was potential errors or inadequacies within the media. We tried making a new bottle of media, the effects were not observable. It was then postulated that the fetal bovine serum (FBS) that we had used may have lost its efficacy to help cells grow and develop. When we supplemented a new bottle of media with new FBS, the cell number grew at an exponential rate. The final issue that was encountered within the lab was the contamination that occurred in specific instances. Although the hood is a sterile environment it is possible for contamination to occur. In most instances we supplemented the media with penicillin/streptomycin to prevent bacterial growth. However, for a brief period we ran out of media and borrowed media from another

professor, this proved to be detrimental as this media did not have the penicillin/streptomycin. This resulted in contamination and bacterial growth that overtook the cells. Bacteria are more equipped to grow and replicate in conditions harsher than the environment that the cells grow in, therefore the bacteria when introduced to an environment with susceptible cells will dominate the environment. The contamination was nearly impossible to avoid with the introduction of the foreign substances, the heating element and wires, and the lack of defensive antibiotics to destroy even one bacterial cell that may have fallen into the media. There were also times when the CO2 ran out in the incubator as well as some flasks not having vented caps, meaning that the researcher had to manually vent the caps. This prevented adequate CO2 and resulted in some cellular death. Finally, there were also some concerns of cellular senescence, which is when the cells have replicated so many times that the materials within the cell no longer function correctly to divide.

Experimental Setup Limitations

Although the secondary experiment had some promising results it was not the primary hypothesis of the experiment. The primary hypothesis was to test whether the potentially cheaply, mass produced heating elements were exposing the vapor, or surrounding media in the experiment, to any heavy metals or reactive oxidative species. The initial experimental setup looked promising with the ability to pass a charge from a 9-volt battery into the heating element removed from a JUUL style vape. However, the first iteration of this used 18-gauge single stranded copper wire which proved to be less than ideal for its flexibility and ability to be attached securely to the heating element. The

heating element in the first iteration was removed from the plastic case, due to concerns of the plastic melting during the autoclave process. This was not the ideal setup as the small heating element was difficult to work with especially with the 18-gauge copper wire. The heating element also fell apart since it was out of the containment unit that it would be present in normal use. In the second iteration of the experiment the heating element was replaced with a new pod that had the juice drained away. The larger copper wire was able to be attached but was not a secure method of attachment. The experiment soon proved difficult as after the setup was autoclaved for sterility purposes, the heating element became unattached to the wires and had to be manually reattached destroying the sterile environment. This resulted in a time delay in the treatment of the first group. Upon treatment, the wires touched each other, preventing the charge from passing into the heating element and shorting out the battery. This was a result from the lack of flexibility in the more rigid copper wire and the inability to bend the wire a certain way to prevent the heating element from becoming unattached. This resulted once again in a time delay and a new system being designed in order to alleviate any further issues regarding the original setup.

The third iteration of this experiment involved the addition of alligator clips that securely attached the heating element to the wires. The wires in the third generation were an 18-gauge multi wire or braided type, which allowed for maximum flexibility to prevent the wires from touching and preventing the heating element from detaching. Unfortunately, the third iteration of the experiment did not work as planned either. It was postulated that the media that may have been left in the heating element after the second trial may have caused a catastrophic failure in the abilities of the heating

element. The media was believed to have been superheated in autoclave and caused irreversible damage to the heating element. The wires also had miniature frays that may have prevented charge from flowing adequately. So, we had to determine another course of action since nothing we had tried to this point had given us any usable data.

We decided that we could attempt to test what effects that the vape juice itself would have on cellular growth and development. Although this was not the main hypothesis to be studied it did help the overall direction of the project. The process of treating the cells with the juice at first was thought to be a simple task, however it was soon discovered that the vape juice was a glycerin-based oil that is not soluble in water. This means that the juice will not mix with the media used to treat the cells. If we treated the cells with this method the cells would just simply not grow in the regions treated and the data would be inconclusive on the effects of the juice. To combat this fact, we placed small volumes of juice into absolute ethanol (100% or 200 proof) which would allow the juice to disperse into the media in an even fashion. At first it was thought that the treatment amounts, in the nanograms per milliliter, would be too low to observe any actual results. But it was discovered that it only takes a few microliters of the vape juice when used within an e-cigarette to elicit a “buzz” response for the trillions of cells within the body. So, the nanograms that we would be using were adequate to treat perhaps a few million cells in the experiment.

If there was more time and I had the ability to develop a better apparatus to treat the media for the analysis of the potential damaging materials that could have possibly been introduced from the heating element. Ideally the analysis that was carried out regarding the vape juice could have also been done with the heating element media.

The two could have been compared or perhaps even ran in tandem to observe what effects both might have on cells. Further experiments could be run to analyze what compounds are present in both the vape liquid, as well as the heating element treatment. With this information it could be postulated what specifically had or could have a mitogenic effect on the cell proliferation rates.

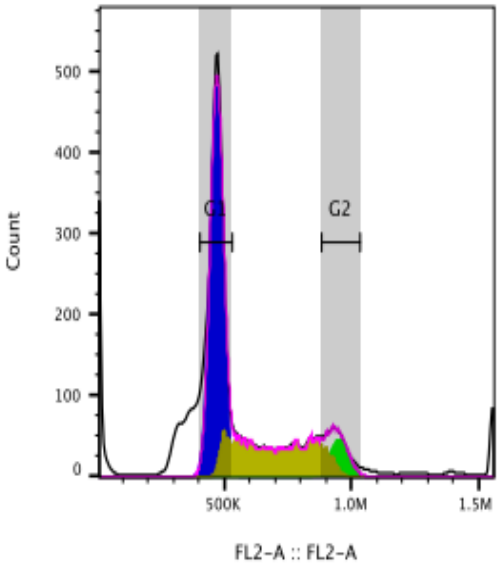
Results

There were a few experiments that were accomplished over the course of this research project. The first two interactions of the first experiments regarding the heating element had significant limitations and produced no usable data. However, the second experiment regarding the vape liquid produced interesting results. The cells exposed to both treatment groups of vape liquid groups displayed a mitogenic effect, with proliferation at significantly higher rates that the control and ethanol treatments groups. Each of the control assays can be viewed in **Figure 2**. The absolute ethanol assays can be viewed in **Figure 3**. The low concentration can be viewed in **Figure 4**. The high concentration can be viewed in **Figure 5.** The mean values of each group can be observed in **Table 1**. The main value of interest in the experiments was the percentage of cells within the S phase. This is important because the S phase is the synthesis phase where cells are duplicating DNA and cellular machinery, such as organelles and protein complexes, to prepare for a mitotic division. Increased S phase values mean that the assays are proliferating at a higher rate than what is deemed normal in perfect growth conditions. The S phase values in the Low treatment had a nearly 150% increase over the control. This would indicate that there was some mitogenic effect in

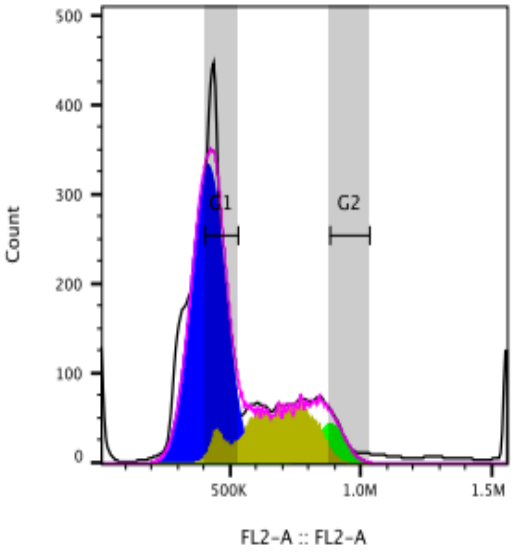
the treatment groups from the vape liquid. Whatever this compound may be, it caused the cells to break from normal protocols and release into rapid proliferation. This is a similar process that occurs in tumorigenesis. The cells are damaged, and the control mechanisms do not function correctly causing the cells to divide uncontrollably. This is not to say that our cells became tumorigenic, however they did display similar characteristics.

Table 1: The mean values of each phase for each treatment group after a 24-hour treatment period.

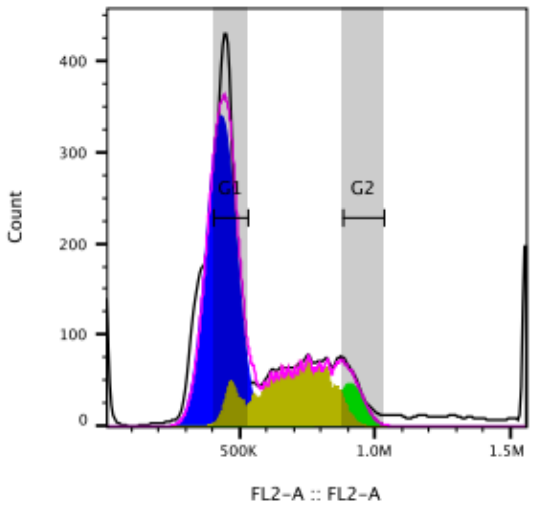
Treatment Group	G1	S	G2
Control	53.07	26.33	6.63
EtOH	39.17	24.93	11.17
Low	27.70	38.40	9.17
High	15.58	15.48	2.96



RMSD : 13.1
%G1 : 42.3
%S : 26.2
%G2 : 6.09
G1 Mean : 4.70E5
G2 Mean : 9.52E5
G1 CV : 7.73
G2 CV : 5.66
% less G1 : 20.7
% greater G2 : 5.27

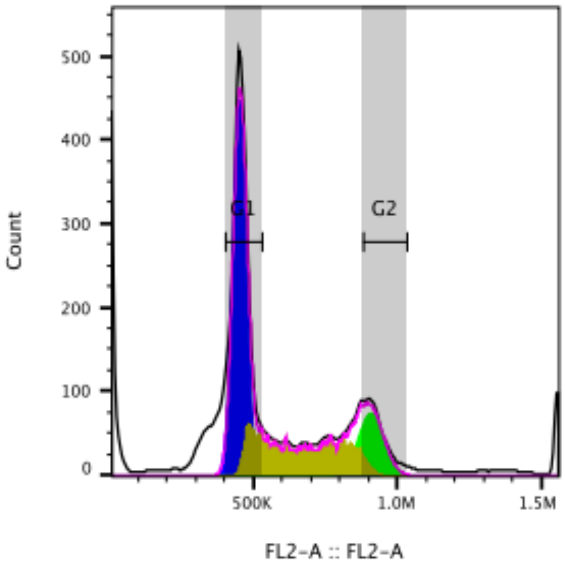


RMSD : 10.1
%G1 : 62.3
%S : 25.1
%G2 : 6.98
G1 Mean : 4.18E5
G2 Mean : 8.86E5
G1 CV : 22.3
G2 CV : 8.82
% less G1 : 4.41
% greater G2 : 6.55



RMSD : 10.8
%G1 : 54.6
%S : 27.7
%G2 : 6.83
G1 Mean : 4.34E5
G2 Mean : 9.10E5
G1 CV : 17.9
G2 CV : 7.66
% less G1 : 5.26
% greater G2 : 9.20

Figure 2: The results of the cell cycle assay for the control group, which had no added treatments. The primary peak of interest is the brown S peak.



RMSD : 14.9
%G1 : 37.1
%S : 25.1
%G2 : 11.7
G1 Mean : 4.53E5
G2 Mean : 9.09E5
G1 CV : 7.34
G2 CV : 6.91
% less G1 : 19.3
% greater G2 : 6.47

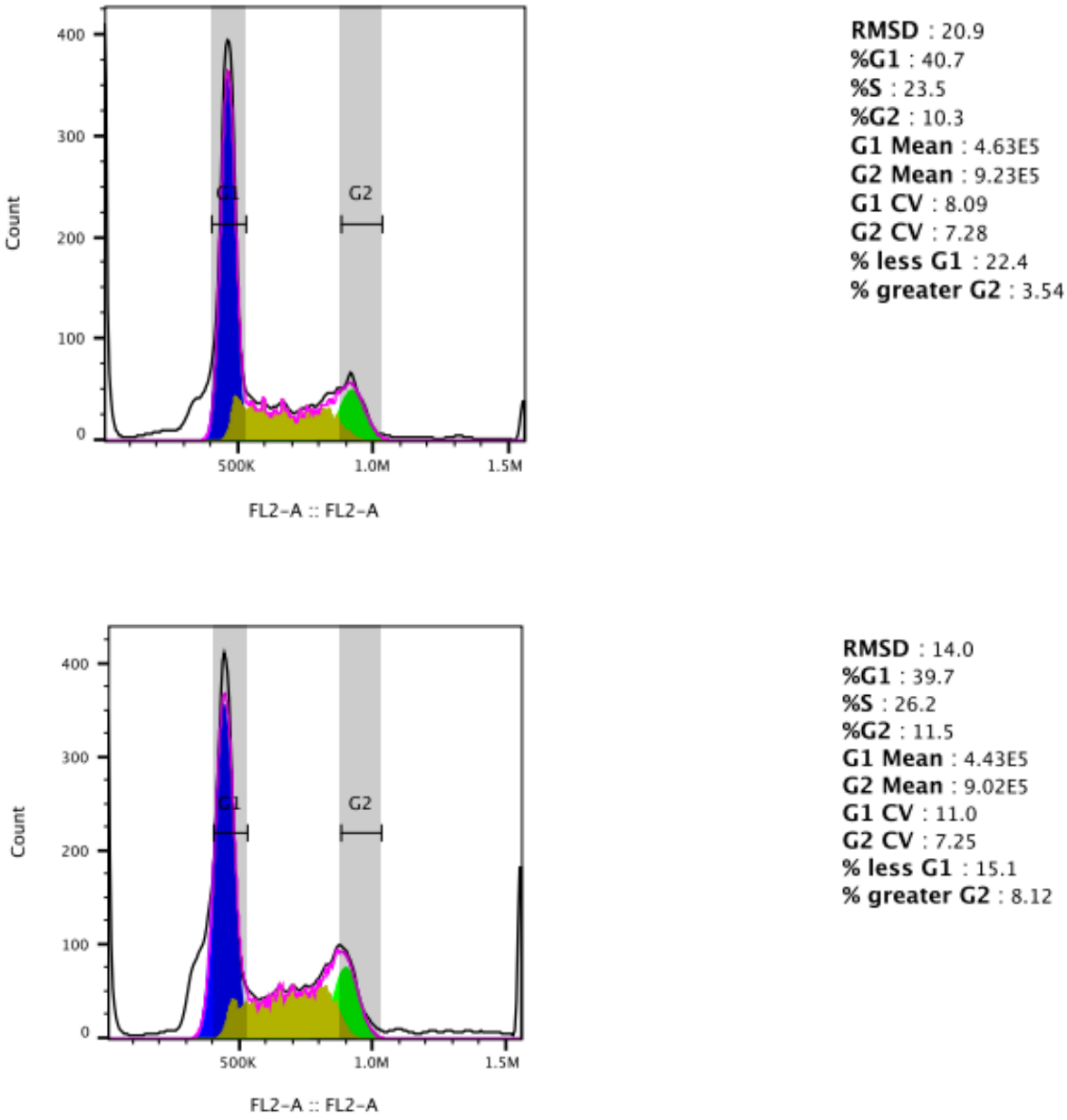
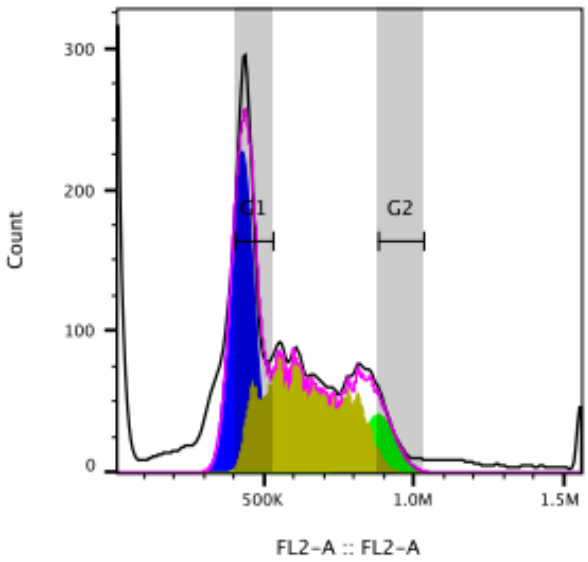
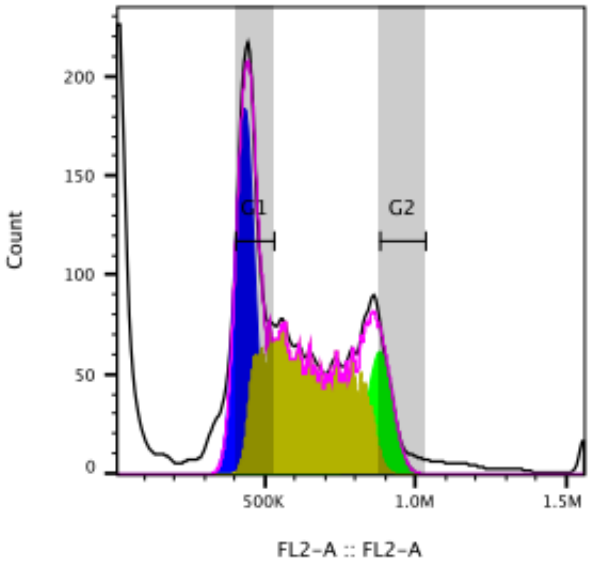


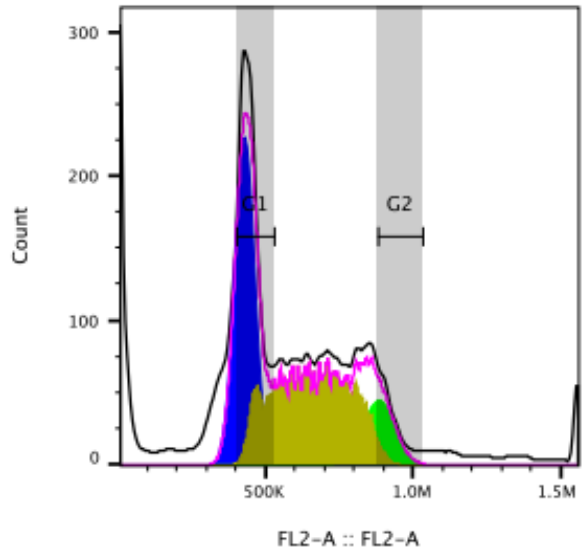
Figure 3: The absolute ethanol cell cycle assay results displayed slightly reduced S phase percentages, but this was to be expected due to the cytotoxicity of ethanol.



RMSD : 16.4
%G1 : 30.1
%S : 37.1
%G2 : 7.93
G1 Mean : 4.28E5
G2 Mean : 8.83E5
G1 CV : 12.6
G2 CV : 8.92
% less G1 : 20.0
% greater G2 : 5.54

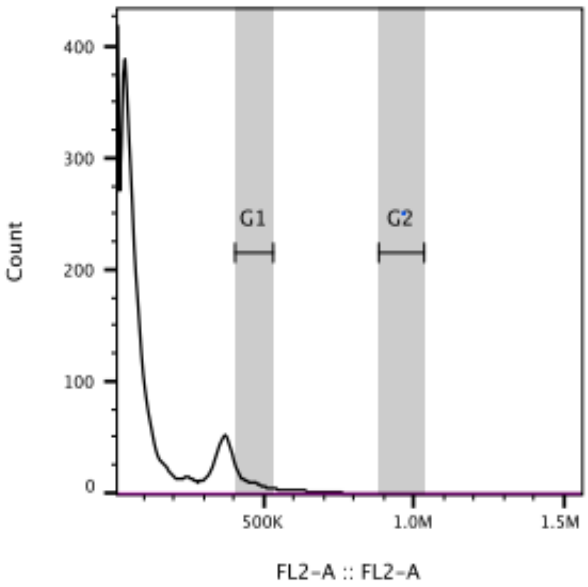


RMSD : 13.8
%G1 : 23.1
%S : 39.4
%G2 : 10.4
G1 Mean : 4.35E5
G2 Mean : 8.83E5
G1 CV : 10.3
G2 CV : 6.74
% less G1 : 23.3
% greater G2 : 3.78



RMSD : 12.5
%G1 : 29.9
%S : 38.7
%G2 : 9.19
G1 Mean : 4.31E5
G2 Mean : 8.85E5
G1 CV : 11.2
G2 CV : 8.42
% less G1 : 16.7
% greater G2 : 6.10

Figure 4: The results for the cell cycle assays for the Low treatment. The Low treatment groups displayed a significant increase of approximately 1.5x the control in the S phase peaks.



RMSD : 99.7
%G1 : 1.65E85
%S : 3.30
%G2 : 0
G1 Mean : 4.01E5
G2 Mean : 9.72E5
G1 CV : 2.42E84
G2 CV : 0
% less G1 : -294
% greater G2 : -577

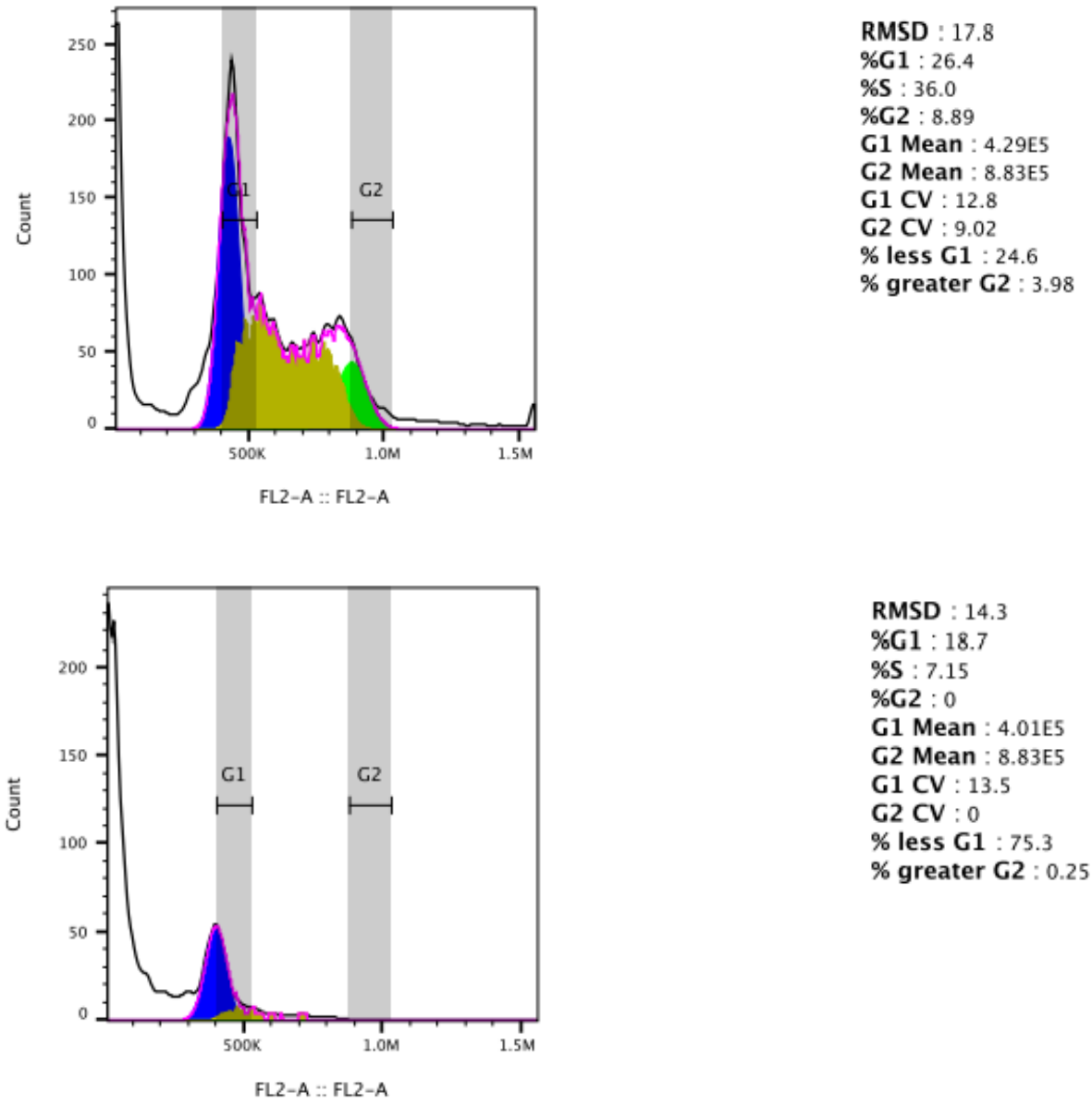
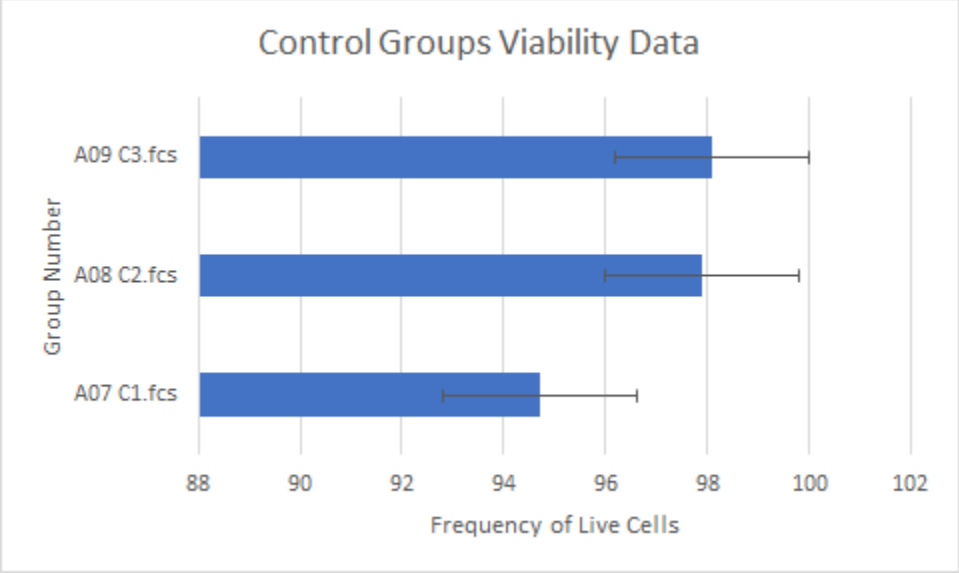
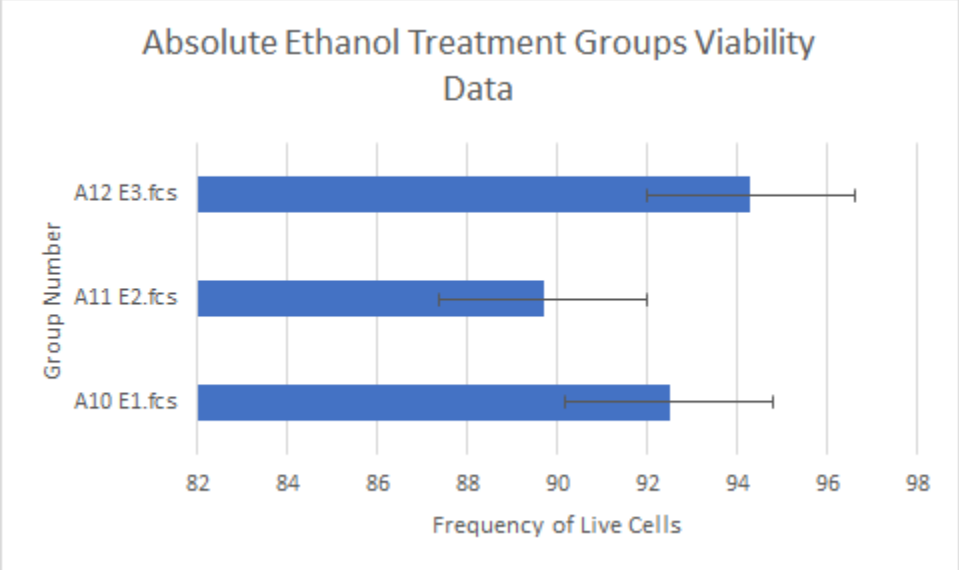


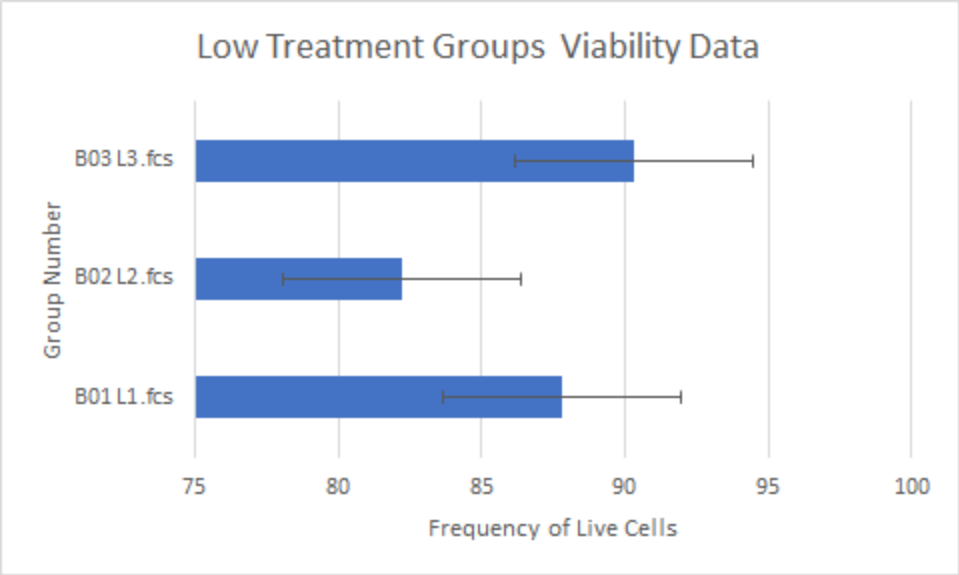
Figure 5: The results of the cell cycle assay for the High concentration. The High concentration did prove to be cytotoxic and killed most of the cells in two of the three assays, however in the third the increase in the S phase peak was also observed.

The high concentration also proved to be heavily cytotoxic and killed the majority of cells in two of the three treatments. The cytotoxicity of each of the treatments can be observed in the cell viability data. The data was obtained via flow cytometry. The

viability data for the different test groups can be observed in **Figure 6**. The viability values give us insight into the ability of the cells to survive and proliferate through the treatment's effects. The averages of the groups can be observed in **Table 2**. For the most part the cell viability remained above 80%, except for during the High treatment where it dropped as low as 36%.







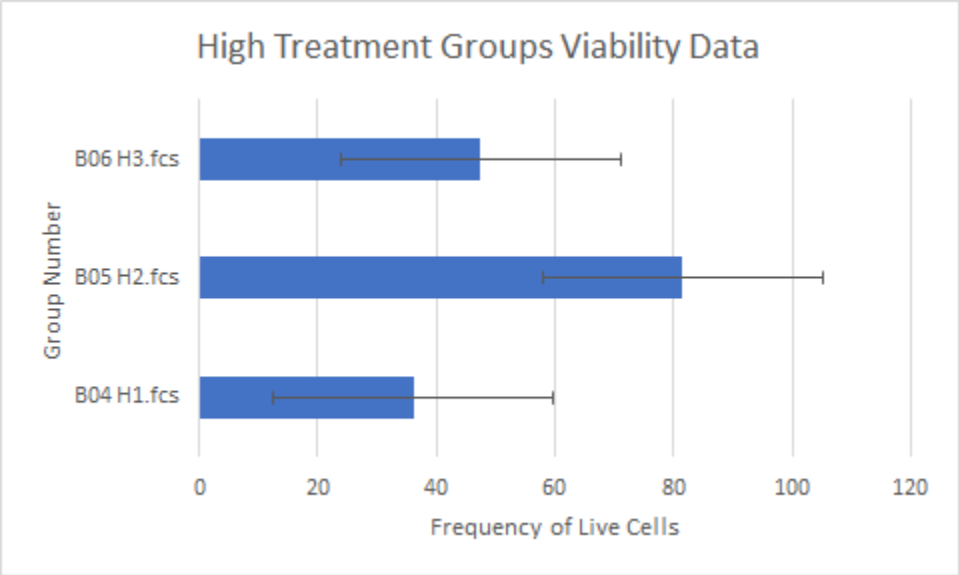


Figure 6: The flow cytometry results demonstrated the relative fitness of the cells present in each test group. It can be observed that the frequency of live cells drops as each treatment becomes more cytotoxic.

Table 2: The mean cell viability data as well as the standard deviation involved within each treatment group. There is heavy variability within the high treatment group as the values vary from 36 to 82%.

Treatment Group	Cell Viability	Standard Deviation
CONTROL	96.9	1.91
EtOH	92.17	2.32
LOW	86.77	4.15
HIGH	55.03	23.59

Literature Review

In *Toxicogenomic approaches for understanding molecular mechanisms of heavy metal mutagenicity and carcinogenicity,* Koedrith et al. demonstrate the adverse effects that result from exposure to heavy metals. The article provides several analysis methods that are useful to determine the extent of cellular damage. These include tasks such as gel electrophoresis, treating cells with antigens to determine levels of enzymatic activation, or to observe biomarker presence in cells.

In *Comparative in vitro toxicity evaluation of heavy metals (lead, cadmium, arsenic, and methylmercury)*, Karri et al demonstrate the several types of damage that can occur including mechanistic potency of DNA damage and apoptosis damage with the observed cytotoxicity. The article's central idea was that experimental data supports that lead, cadmium, arsenic, and methylmercury induce cytotoxic, genotoxic, and

apoptotic effects on HT-22 cells in potency-dependent manner. This demonstrates that these metals can have adverse reactions in certain concentrations. The researchers used the comet assay, annexin-V FTIC / propidium iodide (PI) assay, to analyze the genotoxicity and apoptosis. The results of the cytotoxicity assay clearly demonstrated significant concentration and time-dependent effects on HT-22 cell line. The Annexin V/PI assay is a commonly used tactic for analyzing apoptotic cells. PI is utilized due to its economic value, stability and it is a good indicator of cell viability, based on its capacity to exclude dye in living cells (Reiger et al. 2011). The ability to determine if the cells are going into an apoptotic state is important to understand the extent of the damage to the cell's DNA and machinery. The cell must be damaged beyond a point of repair and the cell will destroy itself to attempt to prevent adverse effects on the body, such as cancer formation. If it is evident that the cells are going into an apoptotic state than there is evidence that the treatment of the oxidative species and heavy metals are damaging the cells in a potential variety of ways.

The researchers examine the evidence for involvement of the oxidative stress in the carcinogenesis process. Attention is focused on structural, chemical and biochemical aspects of free radicals, the endogenous and exogenous sources of their generation, the metal (iron, copper, chromium, cobalt, vanadium, cadmium, arsenic, nickel)-mediated formation of free radicals, the damage to both nuclear and mitochondrial DNA, the damage to lipids and proteins by free radicals, the phenomenon of oxidative stress, cancer and the redox environment of a cell" (Valko et al. 2006). This is important to this research project as it demonstrates in great detail the mechanism of oxidative damage and carcinogenesis. This is essential in order to understand the

mechanisms of cellular damage. This damage can cause the cell to undergo the process of apoptosis, programmed cellular suicide, or undergo carcinogenesis and produce cancer cells. This can occur if the DNA or cellular protectorate machinery is damaged to the point where the cell can no longer control its own growth and development.

Discussion

The low and high-level treatment groups displayed significantly higher replication states which would indicate that there is a mutagenic quality to the juice itself. The higher treatment group also proved to be cytotoxic and killed many of the cells in two of the three test groups. It is suspected that the test groups will display considerable amounts of p53 expression, although this data is not ready to discuss yet. There are several ways that the juice could have mutated the cells, a likely candidate was the presence of heavy metals or other reactive oxidative species. These free radicals can damage and disrupt cellular DNA and machinery alike. The pathways and type of damages that these ROS can take can be viewed in **Figure 7**.

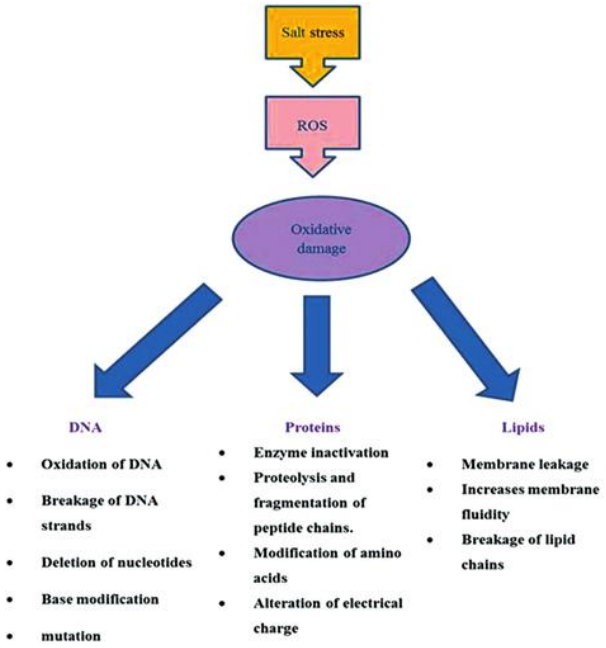


Figure 7: The ROS can affect nearly any cellular component and wreak havoc upon their structure and function. There are a variety of ways they can influence or disrupt the function of DNA, proteins, and lipid membranes alike. (Mushtaq et al 2020).

Heavy metals and oxidative species will react and disrupt intermolecular forces in cellular machinery and DNA. Heavy metal exposure can cause aberrant changes in both genetic and epigenetic factors via non-targeted multiple toxicogenomic technologies of the transcriptome, proteome, metabolome, and epigenome (Koedrith et al. 2013). This quote describes the mechanism of the non-targeted disruption of molecular bonds, the cause of damage to the cell. Because genome sequencing was not run on the cells it is impossible to determine what effects the mitogens present within the vape liquid had upon the cellular DNA. The p53 assay would guide the understanding that of there was high concentrations of p53 it would be reasonable to suspect cellular DNA damage and an attempt to rectify this problem.

P53 serves as a protectorate of the genome. It leads a series of processes that leads to apoptosis and tumor suppression when it functions correctly. It detects DNA or

cellular damage and suspends the cell cycle so the problem can be rectified or terminated. P53 serves by quality checking DNA and cellular mechanisms, if it detects cellular abnormalities, it halts the cell cycle at one of a few different checkpoints. If the problem is deemed irreversible then apoptosis is initiated and the cell will self-terminate via the release of apoptotic enzymes present within the cell, or the alteration and suspension of metabolic processes. Unfortunately, since the p53 protein controls so many safeguards of the genome when it mutates the effects can be catastrophic. If the p53 can no longer accurately bind to DNA, then the suppressor genes that are targeted by p53 for production are not produced. Therefore, the cell cycle is not halted, DNA cannot be repaired as accurately, nor can the cell be signaled for destruction via apoptosis. This creates the perfect environment for the cell to divide regardless of external or internal signals that would usually regulate division. This uncontrolled division along with some inability to repair DNA or commit apoptosis allows for the rapid growth of tumors within the body. P53 is often damaged or mutated in many of the cancers or tumors that are seen today. This vital tool if mutated or nonfunctioning cannot quality check or suspend the cell cycle. This allows cells to proliferate faster, and with many inaccuracies. This is similar to the results we observed in the Low and High treatment groups. Although the p53 data is not yet available, it is postulated that there could be increased levels of p53 to respond to any mutagenic chemicals and damage caused by the treatments to the cells.

Conclusion

Overall, despite the experiments regarding the heating elements were not able to produce any viable data, further research could be done to determine if there are any mutagenic effects associated with the use of the heating element. The second experiment yielded results that indicate that there are mitogens present within the vape juice that caused increased proliferation. Further research could go into the analysis of the vape solution to determine what chemicals inside may have caused the mutations or damages that allowed the cells to proliferate at such a high rate. Better experimental setup could allow for the heating element to be analyzed and determine if there is any risk associated with it, then a tandem study could be done to determine if a combination of both could potentially have an even greater harmful effect. The mitogens present within vape liquid could be mutagenic or carcinogenic in nature, however this cannot be known for sure without further testing. This testing could include the p53 assay kit, to determine cellular expression of the protectorate, or the genetic sequencing to determine what if any of the DNA was damaged via the low and high treatment groups. An improvement in the concentrations of the High treatment group could be made to obtain better results, without terminating the majority of the cells.

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