E-cigarettes: Safe Nicotine Delivery System?

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Abstract

E-cigarettes have become increasingly popular with people throughout the United States. Unlike regular cigarettes, ecigarettes do not produce a heavy smoke, but nicotine is still present. However, there has been a lack of research done on the substances found within liquids that make the e-cigarettes so popular, so more needs to be done. This is a problem because middle and high school aged children are using them without knowing their composition. Therefore, this subject needs to be explored, so we can educate our young people about the possible effects. The present study hypothesizes that there are aldehydes contained in e-cigarettes from nicotine being present, and that the substances will have properties that nick DNA. In order to demystify the youths misunderstanding of their use with vaping products, laboratory experiments were designed specifically to detect aldehydes and the effect that the product could have on DNA. Specifically, a DNA nicking assay was designed to see if DNA would damage from fractionations of e-cigarette solutions, as well as a Purpald solution to detect if any aldehydes were present, and a spectrometer to observe the absorbance. Aldehydes were detected using the Purpald solution procedure, but further research must be done to show conclusive evidence the e-cigarette products do damage DNA.

Introduction

Smoking cigarettes are becoming a thing of the past. Nowadays, people are turning to vape pens or e-cigarettes to fulfill a need or to socialize with their peers. Specifically, to vape means to inhale and exhale the vapor produced by an electronic cigarette or similar device. According to a 2018 report from the CDC on e-cigarette use, 20.8% of high school students and 4.5% of middle school students used e-cigarettes before. Though these small devices are commonly embraced as a healthier alternative than smoking regular cigarettes, they still pose health problems for us. Little do people know that these devices contain toxic chemicals that are detrimental to the body. People think that "vaping" is harmless and pose no potential threats. However, according to Andrey Khlystov and Vera Samburova (2016), "several studies have demonstrated formation of toxic aldehydes in the vapor during vaping." Still people are hooked on the flavorful vapors that are emitted from e-cigarette pens and believe that the liquid within the vapor is harmless. Also, the amount of information available on the flavoring of the liquid in vape pens is scarce, so there is a need to determine whether the flavors that the manufacturers such as: Fairwinds, Alibaba, and DHgate are deemed suffice and safe for users across the board.

The defining mechanisms of this research are important because vape pens have infiltrated elementary schools. With that, the children ranging from 6th grade to 12th grade who have started at an early age not knowing the true side effects of vaping. Without the proper information, and the right message to the younger generations, vaping will only be the start to their smoking journey, and could potentially lead them to experiencing narcotics, and other fatal drugs. Testing the liquids associated in the drugs will determine what is exactly within the drugs and determine which vaping liquids are detrimental to the human body. Thus, the "vaping" phenomena must be deemed as unhealthy until further research has been carried out.

The perception of vaping is something that needs to be justified. No longer should people view vaping as harmless with no side effect. The purpose of this research is to discover the underlying chemicals contained within vaping products that may pose detrimental health concerns on our health.

Background/Literature:

Vaping has roots dating back to the fifth century in a place that is known today as Afghanistan. However, it is known as a modern trend that is continuously taking new strides in the consumption of nicotine. Most recently, E-

cigarettes were introduced in the market and have led to the what we now know to be vape pens that are battery powered electronic devices that deliver nicotine or nicotine-free "vapors" to smokers in aerosol form (Khlystov and Samburova). It is closely related to devices such as hookahs, bonds, but in this modern day in age, people enjoy products that are sleek and small enough to accompany with them along their day-to-day activities. They are operated by a single battery that has atomizers attached to it. In turn, when turned on, the atomizers then heat up the liquid that is within the cartridge creating a vapor that sends the nicotine or flavor of choice to the consumer. The flavoring compounds associated with vaping, and the process that allows the liquid to heat up, decomposes the compounds within the liquid, and in turn leads to the production of aldehydes (Khlystov and Samburova). To clarify, aldehydes are organic compounds formed via the oxidation of alcohols and include formaldehyde (methanol) and acetaldehyde (ethanol). Through the formation of aldehydes, triglycerides are formed, and if high concentrations of triglycerides are found within the bloodstream, the risk of strokes are increased. Therefore, the perspective that vaping is very safe and does not have adverse side effects must be dealt with and addressed to keep our youth and adult populations healthy.

Teens across America, somehow and someway, are purchasing vape pens like they are candy even though the Food and Drug Administration (FDA) has regulated the sale of vape products by banning the sale of people under the age of eighteen years old (Klager, et. al). These teens are obtaining fake identification cards and have easy access to the newfound trend. The ignorance is substantial, and even though children are generally healthy individuals due to age, vaping initiates the decline in one's health if one habitually uses the products. In fact, the American Chemical society believes that, "Smoking is the second leading risk factor for early death and disability in the world." (Korzun, et. al.) This is also a problem for the youth, more so than adults because their brains are not fully developed yet. The prefrontal cortex (PFC) which is responsible for the executive functions and attention functions, has not fully developed in adolescents, so consuming nicotine during that time is a precursor for cognitive impairment later on in life (Goriounova & Mansvelder, 2012). Comparing smoking behavior of adolescents to that of adults may point to an enhanced sensitivity of the adolescent brain to addictive properties of nicotine. According to Colby et al. (2000), "adolescents report symptoms of dependence even at low levels of cigarette consumption." The most susceptible youth lose autonomy over tobacco intake already within 1 or 2 days of first inhaling from a cigarette. Among adolescents the appearance of tobacco withdrawal symptoms and failed attempts to stop smoking can precede daily smoking dependence and appear even before consumption reaches

two cigarettes per day (DiFranza et al., 2007). The popularity vape pens have gained since their arrival to the mass smoking markets proved may be strong indicators for addictions in young adults and teenagers who start vaping at an early age.

Research Question (Thesis) and Rationale:

My research question is "Are aldehyde levels present in e-cigarettes and how do e-cigarettes affect PUC-18 Plasma DNA?" This is important to delineate because adolescent children are being exposed to the potentially harmful toxins, and they might not be aware of the hidden dangers.

Methods/ Research Design:

The purpose of this lab experiment is to test the compounds found in in the e-liquids that go into the chambers that are decomposed to produce a vapor, and the amount of DNA nicking that those compounds may pose. To do that a Purpald solution was used to detect the for aldehydes within the e-liquid. Aldehydes are known to be toxic, primarily by their facile reaction with primary amines and thus their ability to wreak havoc with protein structure. This will be done by adding a drop of the e-liquid to a solution called Purpald, which will then be dissolved in a 2 mL of 1 N solution of sodium chloride. If, detected, the reagent, Purpald, 4-Amino-3-hydrazino-5-mercapto-1,2,4triazole, will then produce purple colored solutions. Depending on the amount of time the solution takes to turn purple, the aldehyde will be defined. We then analyzed the purple solutions by UV/VIS. Based on the UV spectra, we have detected acetaldehyde in both vaping and hookah solutions.

A smoking apparatus was used to mimic the action of inhaling the vapor from the e-cigarette to collect residue. To do this, a 50 mL syringe was used to produce the smoker's action of inhalation and then the contents of that will be collected onto cellulose filters. Then the residue collected will be removed from the filter using acetone or water and then dried down by a Centrivap vacuum. Then the filter will be suspended in water once again and fractionated with a sephadex.

Furthermore, a DNA nicking assay was be used to determine the possible DNA damage from the use of ecigarettes. That assay uses the properties of supercoiled PUC-18 DNA which will be assorted with different amounts of e-liquid, and accompanying controls. Then those mixtures were loaded into the wells of agarose gel containing either the e-liquid and PUC-18 DNA, PUC-18 DNA itself or the glucosamine control. Determinately, if the DNA has been nicked, the nicked DNA will be much lower on the agarose gel than DNA that was intact. The glucosamine is used juxtaposed to the PUC-18 because it is known to have DNA nicking properties. Finally, ImageJ was used to show the exact amount of nicking that has taken place in clearer view. The brand of the e-cigarette unit was chosen upon availability and popularity. The e-cigarette used was a Blow One T2 2.4 mL blow tank. Instead of rebuilding an atomizer, the stock atomizer will be used for this experiment. Below is a diagram of the apparatus used to contain and collect hookah and vape smoke residue. Every thirty seconds, 30 mL of air is pulled through the Blow One T2 2.4 E-cigarette into a syringe, before being vented to the atmosphere. The smoke is then collected on a cellulose filter, and this first-hand smoke exposure. The apparatus used can be seen in the following image.



A. E-Cigarette B. Cellulose Filter C. Airway D. Syringe

Results/Conclusions:

The mainstream vapors from the e-cigarette were collected on Cambridge filters; the filters were soaked in water and then the aqueous solutions were dried via vacuum centrifugation. After weighing the dried mass, the samples were reconstituted with water and then analyzed by UV spectroscopy; the e-cigarette had an absorbance of (200 Nm). The vaping samples had a peak wavelength of 345.0 nm and an absorbance of 1.130 nm.



We did, however, detect aldehydes in the e-cigarette samples using the Purpald Assay. Aldehydes are highly reactive with DNA, and therefore are mutagenic and carcinogenic. After many years of smoking, the damage caused by aldehydes could accumulate in lung tissue and cause serious illness.



The amount of nicking by the e-cigarette solution is orders of magnitude less that the nicking we have detected with cigarette smoke. However, we have no definitive evidence yet. We had hypothesized that the e-cigarette samples would cause DNA nicking like the gel shown below in wells three and eight of a regular cigarette tar.



Alexander Skochko (2018)

Future work

More work needs to be done on this matter. More products and flavors should be examined as well, so that, our youth would know what exactly are in the products. The CDC has started to express their concern for the use of e-cigarettes and the various marketing strategies that are being produced, so their concern will more than likely spark deep interest on the subject at hand.

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