

Free Radical Production in the Smoking of E-Cigarettes and their Possible Effects in Human Health

Abstract

Background: The increase in smoking e-cigarettes with nicotine or only flavoring compounds requires a deep study on consequences on human health. This research aims to study the possible process, to form free radicals or the nuclei's damages with consequent micronuclei's formation. **Methods:** The study analyzed three groups: the first one that uses e-cigarettes with nicotine, (e-nicotine), the second flavoured liquid (e-vapor) and third the not-smoking group. We determined the salivary malondialdehyde (MDA), the total salivary mucins (SM), and in buccal smear cells the micronuclei (MN). We statistically analyzed the results with the Mann-Whitney U Test Calculator. **Results:** Smoking e-cigarettes e-nicotine or e-vapor produced a great and significative amount of MDA vs control group: $p \leq 0.05$. Only those smoking e-nicotine, have a highest and statically significant amount of salivary mucins vs control group: P value 00496. In both smokers groups, the mean of MN scores has a significant difference vs control group $P \leq 0.05$. **Conclusions:** This study shows the possible damages of the nuclei, but the increase of radicals, oral mucins and MN needs more researches.

Keywords: *Electronic nicotine delivery systems, free radicals, neoplasm*

Introduction

The electronic cigarette is a device designed to simulate and replace both in use and in appearance the traditional cigarettes. Electronic cigarettes may or may not contain a variable measure of nicotine or only flavouring compounds. A commune mixture comprehends water, propylene glycol, glycerol, flavoring compound and or nicotine. The mixture passes from the liquid to the vapor state the atomizer of the device this process don't has the combustion phase and don't produce toxic residues such as tar or polycyclic hydrocarbons. In spite of this, the benefits and probable risks associated with these devices are much discussed. Further, for the relative novelty of this technology, the laws and drug delivery policies public health surveys and the laws governing the sale and use of electronic cigarettes are now the subjects of heated debate in many countries. The scientific knowledge on the efficacy of the electronic cigarette as a quit for smoking cessation or on its potentially harmful effects is still incomplete. It is even clear that e-cigarettes can represent a substitute for the ritual

aspects related to smoking gestures This helps the smoker to contain the consumer of cigarettes. Anyway, it is difficult from the literature data to check this single aspect like an aspect behavioral because the use of e-cigarettes could even present a way of administration of illicit substances.^[1] The aspect related to the impact of the use of e-cigarettes on human health is much more complex. Some liquids may contain more or fewer high concentrations of pharmaceutical nicotine and various compounds present in the formulations currently marketed have toxicological effects in this time unknown. The e-cigarettes may contain nicotine in a variable the amount, (typically, between 2 and 20 mg), mixed with water, propylene glycol, glycerol, and other substances, including flavoring agents. Some models contain no nicotine, but only a liquid, after vapor flavored with many compounds, of which many investigations are underway to set up the real toxicity. In fact, in the literature, only on PUBMED, using as a search key, "e-cigarettes health review", identifies 240 items The main context of our research, however, intends to analyze only the problem related to the probable formation of free radicals during the use

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of e-cigarettes, regardless of whether the liquid has about nicotine. These compounds are important, for their very dangerous consequences, even in the long-term on human health. In this context it was very interesting to analyze, the very recent review, the year 2018,^[2] performed by the “US National Academies of Sciences, Engineering and Medicine Division”, on the consequences for Public Health of E-Cigarettes. This review reports a few studies,^[3-7] to understand their impact on human health, linked to the free radicals’ formation. They are highlights some important aspects

1. There was enough findings that highlight how the components of e-cigarette aerosols could promote and form much reactive oxygen species/oxidative stress. Anyway, the studies show that reactive oxygen species and oxidative stress induction very is lower in the e-cigarettes than from combustible tobacco cigarette smoke.
2. There is real evidence that some chemicals present in e-cigarette aerosols (e.g. formaldehyde, acrolein) are capable to cause the DNA damage. This supports the biological possibility that this could increase the risk of cancer and adverse reproductive outcomes.
3. There is no evidence available that e-cigarette using causes of intermediate cancer endpoints in humans.
4. The most important conclusion of this review, however, shows that in e-cigarettes, the potential risks have less biological activity both in *in vitro* in animal and or human systems.

The bibliographic data confirm that e-cigarettes to produce a variable amount of free radicals. Anyway is not enough to clear if this source of oxidative stress can damage the nucleus of cells as those of oral cavity. This research therefore proposes to study this biological phenomenon with to observe the possible increase of MDA’s amount, the salivary mucin’s concentration and the number of the micronuclei (MN) in exfoliated buccal cells. The MN is small nuclei formed during the anaphase by condensation of fragments of acentric chromosomes or whole chromosomes that are not incorporated into the main nuclei of the daughter cells. Two different types of genetic damage are responsible to form the MN: a) clastogenic agents fragment the cells b) the damage of the mitotic spindle or the centromere of whole chromosomes by aneuploidogenic agents. The micronuclei contain fragments acentric (a clastogenic mechanism) or whole chromosomes (an aneuploidogenic mechanism) produced in migration delays during anaphase. The use of the technique of count the MN as a measure of cytogenetic damage in peripheral blood lymphocytes is since 1976. Genetic damage generally forms the MN and is possible to assess this damage by assigning a score (number of MN) as its indicator. There are two main mechanisms that lead to forming MN in a mitotic cell: the first concerns the chromosomal breakage due to clastogenic agents, while the second is the damage

of the mitotic process due to aneugenic agents which prevent spindle formation during mitosis. As a result of it, there is a retard on to form the chromosomes in anaphase. and with the daughter cells that may have micronuclei containing whole chromosomes. There are also other cases to form MN and what we can see spontaneously in normal healthy people subjected such as to environmental exposure to pollutants radiation bio-hazard materials drugs and poisonous chemicals. Other causes^[8] generate MN as the chronic inflammation, heavy metal poisoning, the chemotherapy radiation injuries, and various pre-cancer conditions: All these causes produce direct damage to DNA or breakage chromosomal aberrations, malfunctioning mitotic apparatus, and interference with DNA synthesis. The protocol correlates the genetic damage to many and various diseases. It clears therefore as well as cancer conditions induce to form many MN and is an aim of this study to verify this possibility in e-cigarettes smoking

Methods

The study provides to select three groups of seventeen people all men with an age range between thirty-two and forty-one years old. The first group (e-Nicotine) smokes e-cigarettes with liquids commercially defined “cloud-chasing” with the commercial name (Old Tobacco Vaporart). The liquid has as components 70% of vegetal glycerine, 30% of propylene glycol, 1% aromatizing compounds and pharmaceutical nicotine equal to 10 mg/10 ml bottle. The second group (e-Vapor) smokes the same type of liquid without nicotine. The third group (control) is the non-smoking group (CG). The protocol excludes people with oral and cardiovascular diseases or diabetes. All people of the first two groups have ceased to smoke normal cigarettes to at least three years. In this study, the volunteers use types of e-cigarettes each different other by brand but not for the combustion device with a battery with a voltage of 75 watts. The protocol provided to determinate the MDA, the Total Salivary Mucins and the MN after that the volunteers have consumed the 10 ml in the bottle. The time to consume a single bottle (10 ml) is two weeks. We determine the amount of salivary malondialdehyde with the Thiobarbituric test and the total salivary mucins with Alcian Blue method. The test for the MN score in exfoliated buccal cells is easily executed rapidly without the need for cell culture and the cytogenetic assay. Volunteers wash their mouth carefully before to collect the buccal mucosa cells. We share the buccal mucosa cells by a wet spatula and distribute the same on small clean glass slides. After fixation by Path fix sprays and drying at room temperature, we use the Papanicolaou staining method to check the cells containing micronuclei. Have examined the cells under an optical microscope (Nikon Ys-100) at $\times 400$ magnification and we have used the criteria of many authors to check the micronucleus. Have counted a total of 500 cells for each sample and we have reported the percentage of the MN. We

statistically analyzed the results with the Mann-Whitney U calculator a nonparametric test. This allowed two groups or conditions compared without making the assumptions that are normally distributed

Results

Below we report the statically analysis of the results. The salivary concentration of MDA is in nM/ml [Table 1], those of total Mucins [Table 2] in mg/dL, while the MN value is the score with standard deviation based on the average number observed.

Results for Table 1:

Average values MDA (nM/ml)

e-nicotine = 3.14; e-vapor = 3.11; control = 2.77

1-MDA e-Nicotine - vs e Vapor. The U-value is 135.5. The critical value of U at $P < .05$ is 87. Therefore, the result is not significant at $P < .05$. The Z-Score is 0.29277. The P value is. 0. 77182. The result is not significant at $P < .05$.

2- MDA e-nicotine – vs Control. The U-value is 61.5. The critical value of U at $P < .05$ is 87. Therefore, the result is significant at $P < .05$. The Z-Score is 2.84159. The P value is. 00452. The result is significant at $P < .05$.

0.3--MDA e-Vapor vs Control. The U-value is 70.5. The critical value of U at $P < .05$ is 87. Therefore, the result is significant at $P < .05$. The Z-Score is 2.5316. The P value is. 0114. The result is significant at $P < .05$

Results for Table 2:

1-Mucins e-nicotine vs e- vapor. The U-value is 62.5. The critical value of U at $P < .05$ is 87. Therefore, the result is significant at $P < .05$. The Z-Score is 2.80715. The P value is. 00496. The result is significant at $P < .05$.

2-Mucins e-nicotine vs control. The value of U is 26.5. The Z-Score is -4.04712. The P value is $< .00001$. The result is significant at $P < .05$

3-Mucins e-vapor vs control: The U-value is 123. The critical value of U at $P < .05$ is 87. Therefore, the result is not significant at $P < .05$. The Z-Score is -0.72331. The P value is 0.47152. This result is not significant a $P \leq 0.05$.

Results for Table 3:

1- MN Scores e-nicotine vs control

The value of U is 22. The z-score is 4.20211. The P value is $< .00001$. The result is significant at $P < .05$

2- MN Scores e-vapor vs control

The U-value is 42. The critical value of U at $P < .05$ is 87. Therefore, the result is significant at $P < .05$. The z-score is 3.51324. The P value is. 00044. The result is significant at $P < .05$.

3- MN Scores e-nicotine group 1 ≥ 39 years, vs e-nicotine group 2 ≤ 36 years *

The U-value is 3. The critical value of U at $P < .05$ is 5. Therefore, the result is significant at $P < .05$. The z-score is 2.32186. The P value is. 02034. The result is significant at $P < .05$.

4- MN Scores e-nicotine group 2 ≤ 36 years, vs control group group 1 ≤ 36 years *

The U-value is 1. The critical value of U at $P < .05$ is 8. Therefore, the result is significant at $P < .05$. The z-score is 2.93883. The P value is. 00328. The result is significant at $P < .05$.

*Note: The approximation to the form of the normal distribution becomes less robust at sample sizes smaller

Table 1: salivary MDA concentration in three groups

e- nicotine age years	MDA nM/ml	e-vapor age years	MDA nM/ml	No smokers age years	MDA Nm/ml
32	3.0	32	2.9	33	2.1
33	2.9	34	2.9	34	2.7
33	2.8	34	2.8	35	2.4
35	2.8	35	2.6	35	2.6
35	2.8	36	2.9	35	2.5
36	2.9	36	2.9	36	2.7
36	2.8	37	2.8	36	2.7
37	3.0	37	2.8	37	3.3
37	3.0	37	3.2	37	2.0
38	3.7	37	3.6	38	2.7
37	3.0	38	2.9	38	2.8
39	3.1	38	3.2	39	2.9
39	3.5	39	3.4	39	3.0
40	3.5	39	3.4	40	3.4
40	3.4	40	3.4	40	2.9
40	3.1	40	3.4	40	2.9
41	4.1	41	3.7	41	3.3

Table 2: Total Mucins Concentration mg/dL in three groups

Salivary mucins mg/dL		
E NICOTINE	E VAPOR	CONTROL
36.1	33.0	33
34.0	33.8	33
34.8	33.9	34
35.3	32.9	33.8
35.5	33	33.8
33.0	32.2	33.0
31.0	32.6	33.2
33.0	31.7	32.2
35.4	31.9	32.6
35.0	31.2	32.4
34.0	30.5	32.1
32.5	32.9	31.4
35.3	30.	31
30.4	30.5	31
30.0	30.8	31.5
33.0	32.7	31.7
35.9	32.2	31

than 10, so caution is appropriate here in making use the Z-value calculation.

Discussion

This study confirms the results obtained in other research that show that the use of electronic cigarettes exposes consumers to highly reactive ROS. These studies show that in the device of e-cigarettes, the heating element when vaporizes the various compound forms a high amount of ROS. In our study, the liquid that contained nicotine” OLD TOBACCO “as formulation “and the liquid with only glycerine, propylene glycol and or the flavoring compound produce the same amount of MDA: P value = 0.77182. The salivary MDA, see Table 1, in both cases has anyway significant higher concentrations vs the control group: the P values are <0.00452 and is 0.114. A recent EPR study,^[4] by using as the spin-trap the phenyl-N-tert-butyl nitron (PBN), shows that the aerosol phase generated radicals from both propylene glycol and glycerol, but also by the PBN radical adduct. This fact is to show as the free radicals may have different chemical origins. In another study,^[7] the e-cig vapors have formed about 7×10^{11} free radicals for puff and elicited to a significant increase in oxidative stress. This amount of free radicals represents an amount that is certainly toxic to cells. This fact anyway wasn't completely understood because theoretically that e-cig vapor does not contain any combustion products. Realty the heating element in some conditions produces radiation, and furthermore, the battery output voltage could generate toxic chemicals. The age of the heating element influences this process^[6] and further the lithium-ion battery of the device may form the oxidant compounds. A recent study^[8] shows as the

aerosol phase produces the free radicals and in particular is the chemical reactivity of glycerol and propylene glycol to promote this process. Other studies have also indicated that^[9,10] the flavoring elements produces in some conditions high amount of free radicals. The results of this study confirm these conclusions and don't is a statistically significant difference among e-nicotine group vs e-vapors to produce the MDA. It is important to note, however, that the results of this study, (see Table 2) show that in the e-nicotine group, there is a statistically significant increase of salivary mucins versus the other two groups. Theoretically the amount of mucins should decrease at the same time as the increase in free radicals. because as previous reports show the free radicals degrade all proteins^[11] and change the mucins in their most important fractions, sugar and protein moieties.^[12,13] The results of this research have a possible explanation if we hypothesize that nicotine in these experimental conditions promotes a pro-inflammatory process to salivary glandular apparatus with later hypersecretion of mucins. This hypothesis apparently contrasts with the results of an other research,^[14] that show that in the tobacco smoking and much more in chewing, the nicotine does not interfere with epithelial cells of the oral mucosa. Nicotine is in fact a weak base with a pKa of 8.0 and its ionized state in acidic environments does permit not rapidly cross membranes. The pH of smoke the tobacco present in most cigarettes, is acidic (pH 5.5–6.0). At this pH, nicotine is primarily ionized, and the consequence is that the same there has a little buccal absorption.^[15] Smoking the e-cigarettes that contain nicotine without tobacco instead promotes the nicotine to interact with saliva that normally has a neutral or slightly basic pH. This condition facilitates the across of nicotine into the buccal epithelium and stimulates the glandular secretion for the mucins. A similar phenomenon happens for the mucus in the pulmonary alveoli.^[16] of tobacco smokers. The rapid absorption of nicotine from cigarette smoke through the lungs presumably is two of the largest surface area of the alveoli and small airways, and dissolution of nicotine in the fluid of pH 7.4. This biological status in the human lung ease transfer across membranes of the mucins. In this study, the amount of salivary mucins increases in the e-nicotine group versus to e-vapor group with the P value 0.0046 and vs control with the P value 0.00001, [Table 2]. This transfer across of nicotine in oral membranes so increases mucin's production and causes a greater viscosity of mucous. The increase of mucus produces an more highest number of terminal glucosides acid groups especially sialic acid that for their hydrolysis promote the pH decrease and downgrades the value of the spinnbarkeit the parameter [Figure 1]. The value of spinnbarkeit measures the adhesivity of saliva to the oral mucosa and the consequence is a lack of the defense of the oral cavity itself. In this study, we have also evaluated the role of e-cigarettes to form the micronucleus and so see

the eventual chromosomal damage caused by genotoxic e-cigarettes components. The micronucleus in oral buccal cells is considered to be a biomarker and induction of micronucleated cells by carcinogens and mutagens is to sign the genotoxic effect of such substances. Saliva soluble compounds present in tobacco could diffuse into the basal cell layer and modify the reproductive mechanism of the underlying proliferating cell population, thereby causing genotoxicity to form many nuclear aberrations. Numerous studies have analyzed this process and showed the correlation between pre-cancerous situations in habitual tobacco smokers and high presence of micronuclei in exfoliated buccal cells.^[17-19] Generally, the distribution of average frequency in the smoking group has a mean which ranges from 6 to 20% of MN in the smokers, while in patients with pre-cancerous situations it can also double. In our study, see Table 3 the results show a score of MN with a maximum value of 3.4 and with a mean of 2.6%. These values are anyway statistically highest respect to the control group *P* value are <0.00001, and 0.00044, respectively. These results indicate that the smoking of electronic cigarettes involves some risks of a carcinogenic nature. However, it is interesting to note that in both groups of e-cigarettes smokers, there are some volunteers in whom the MN score is higher. These people are those who have a higher age ≥ 39 years, see Table 4, and have smoked many more cigarettes, and for which there is also the presence of three micronuclei, see Figure 2. This score still represent a number of genetic alterations much more relevant with respect to those found in their groups people. These values show as is possible the enhancement of the effects of the e-cigarettes after tobacco smoking. Furthermore, this frequency of MN indicates that it takes a long time and not just a few years to dispose of the all the harmful effects of the compounds present in tobacco.

Conclusions

Smoking e-cigarettes does not involve an expression of a state of biological oxidative stress which damaged the normal cellular metabolism and that may conduct to

promote cancer. Even the low number of MN indicates a negligible genotoxic alteration by the components of the

Table 3: MN score in the e-cigarettes groups vs control

e-nicotine group		e-vapor group		Control Group	
Age	MN Score	Age	MN Score	Age	MN Score
32	0.81	32	0.31	33	0.31
33	1.30	34	1.28	34	0.50
33	1.37	34	1.49	35	0.77
35	1.46	35	0.56	35	0.46
35	2.59	35	1.69	35	0.69
36	1.41	36	0.71	36	0.71
36	1.70	37	1.61	36	0.91
37	0.78	37	1.77	37	0.78
37	1.22	37	1.02	37	0.62
37	1.59	37	1.44	38	0.79
37	0.72	38	2.52	38	0.82
39	1.99	39	1.99	39	0.81
39	2.41	39	2.91	39	0.77
40	2.67	39	2.89	40	0.81
40	2.98	40	2.68	40	0.78
40	2.41	40	2.29	40	0.89
41	3.40	41	2.57	41	0.76
Mean	Mean MN	Mean	Mean MN	Mean	Mean MN
36.88	1.81±0.80	37.06	1.75±0.96	37.24	0.72±0.05
years		years		years	

Table 4: MN score in the age subgroups

e-nicotine group 1		e-nicotine group 2	
Age years	MN number	Age years	MN number
39	1.99	32	0.81
39	2.41	34	1.30
40	2.67	35	1.37
40	2.98	35	1.46
40	2.41	36	2.59
41	3.40	36	1.70
Mean years	Mean MN	Mean years	Mean MN
39.83	2.64	34.66	1.54

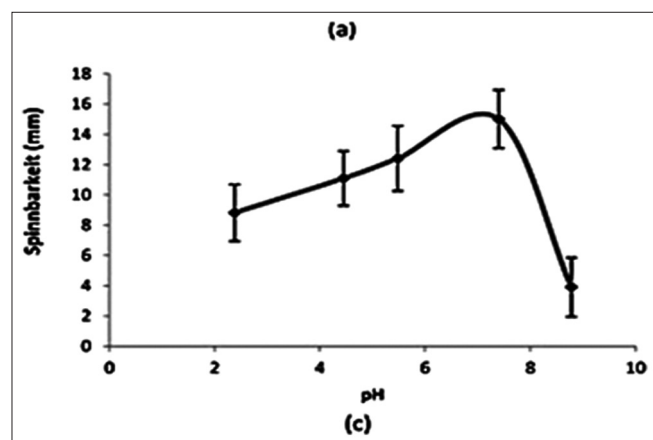


Figure 1: Spinnbarkeit values for the pH index

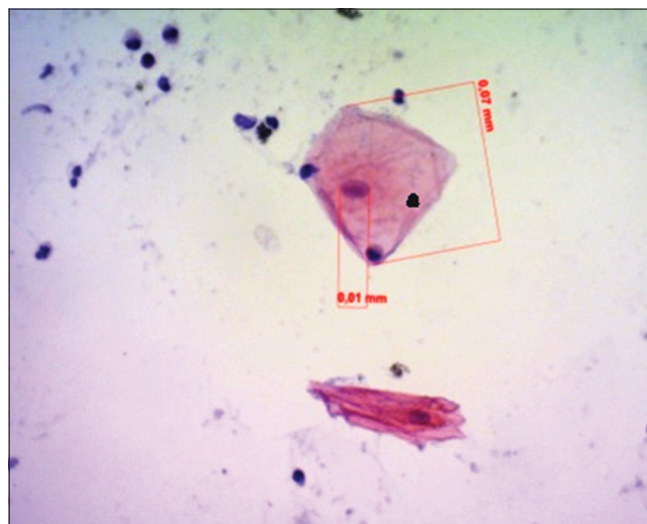


Figure 2: Micronuclei in e-cigarettes subgroups with age ≥ 39 years

various types of electronic cigarettes. These devices contain propylene glycol, glycerol with or without nicotine and flavoring agent, and these compounds don't have cancer properties. Anyway, this study has shown an important salivary mucins increase that causes a greater viscosity of salivary mucus with a consequent lesser defense of the oral cavity itself. In any case, the question to use the e-cigarettes is very complex, and the importance of this topic requires more and more research on both the short- and long-term effects on the health for the consumer. Therefore, the last question is concerning the balance of the costs-benefit ratio, between their effects on the initiation and cessation of combustible tobacco product use, and that will bring clarity to the question of whether e-cigarettes will prove to reduce harm or induce harm at the personal and the population levels. E-cigarette product marketplace and user population are changing, and there will undoubtedly be new issues, which are at this time unknown, and which will need careful surveillance and scientific scrutiny in the future.

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Conflicts of interest

There are no conflicts of interest.

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