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Impact of electronic cigarette heating coil resistance on the production of reactive carbonyls, reactive oxygen species and induction of cytotoxicity in human lung cancer cells *in vitro*

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Abstract

Electronic cigarette (e-cigarette; e-cig) use has grown exponentially in recent years despite their unknown health effects. E-cig aerosols are now known to contain hazardous chemical compounds, including carbonyls and reactive oxygen species (ROS), and these compounds are directly inhaled by consumers during e-cig use. Both carbonyls and ROS are formed when the liquid comes into contact with a heating element that is housed within an e-cig's atomizer. In the present study, the effect of coil resistance (1.5 Ω and 0.25 Ω coils, to obtain a total wattage of 8 \pm 2 W and 40 \pm 5 W,

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Author Contributions

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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respectively) on the generation of carbonyls (formaldehyde, acetaldehyde, acrolein) and ROS was investigated. The effect of the aerosols generated by different coils on the viability of H1299 human lung carcinoma cells was also evaluated. Our results show a significant ($p<0.05$) correlation between the low resistance coils and the generation of higher concentrations of the selected carbonyls and ROS in e-cig aerosols. Moreover, exposure to e-cig vapor reduced the viability of H1299 cells by up to 45.8%, and this effect was inversely related to coil resistance. Although further studies are needed to better elucidate the potential toxicity of e-cig emissions, our results suggest that these devices may expose users to hazardous compounds which, in turn, may promote chronic respiratory diseases.

Keywords

e-cig; resistance; oxidative stress; carbonyl compounds; lung cancer cells

1. Introduction

Electronic cigarettes (e-cigarettes, e-cigs) have been on the consumer market for almost a decade and are marketed as an alternative to conventional combustion cigarettes. E-cigs have been promoted as a potential approach to aid in smoking cessation (Franks et al., 2018). Of particular concern, e-cig popularity has grown rapidly among young people worldwide. In 2014, e-cig use among US adolescents surpassed the use of conventional cigarette for the first time (Arrazola et al., 2014). While e-cigs do not produce carcinogenic combustion products such as polycyclic aromatic hydrocarbons, and e-cig liquids do not contain tobacco associated carcinogens (e.g., nitrosoamines), there is growing evidence that e-cigs do generate harmful substances during their use. Several authors have reported the presence of reactive carbonyls, including formaldehyde, acetaldehyde, acetone and others, in e-cigarette vapor (Goniewicz et al., 2014; Ogunwale et al., 2017; Bitzer et al., 2019). In addition, both stable, long-lived radicals (Lerner et al., 2015a; Lerner et al., 2015b; Sussan et al., 2015) and short-lived, highly reactive radicals (Goel et al., 2015) have been found in e-cig vapor. Oxidative stress induced by cigarette smoke has been shown to play a key role in the pathogenesis of cancer (Pryor et al., 1997), cardiovascular disease (Messner and Bernhard, 2014), and chronic obstructive pulmonary disease (COPD) (Centers for Disease Control and Prevention, 2010; Domej et al., 2014; Kirkham and Rahman, 2006). Likewise, e-cig vapor can induce oxidative stress that can lead to inflammation (Lerner et al., 2015a; Muthumage et al., 2018; Scott et al., 2018), cytotoxicity *in vitro* (Scott et al., 2018; Vasanthi Bathrinaraynan et al., 2018; Scheffer et al., 2015; Zhanh et al., 2012) and toxicity *in vivo* that may increase cancer risk (Canistro et al., 2017).

In contrast to conventional cigarettes, which differ primarily in terms of nicotine content, tobacco variety and filter type, e-cigs are highly customizable with respect to their operating parameters and the chemical composition of their liquids. These liquids are available in a wide-variety of flavors and nicotine levels, and e-cig devices can be programmed to achieve variable power output and variable heating coil resistance levels. Unlike conventional cigarettes, the possibility to choose among such different flavors (e.g., fruit, dessert,

tobacco) is particularly appealing to adolescents and young adults (Harrel et al., 2017; Yingst et al., 2017).

In most cases, modern e-cig devices consist of a mouthpiece, a refillable cartridge, a lithium battery and a heating atomizer. Users activate the heating coil in the atomizer by depressing the device's power button during inhalation; thus, the flavored liquid is rapidly vaporized by passing through the heating element. With new generation devices, users can modulate the e-cig liquid vaporization process by selecting atomizers with different coil resistances, by applying different voltages across the coils, and/or by controlling the operating temperature of the atomizer. According to recent reports, the design and operation settings of e-cigs may have a significant impact on human health (Chausse et al., 2015). Contrary to the initial hypothesis that voltages higher than 3.3 V and up to 5 V are responsible for formaldehyde generation in e-cig aerosols (Jensen et al., 2015), it is now well established that formaldehyde is produced even under lower powered, breath-activated devices (Bitzer et al., 2019). Most of the hazardous carbonyls detected in e-cig vapors are produced during the thermal decomposition of vegetable glycerol (VG) and propylene glycol (PG), the major chemical constituents of most e-cig liquids. In a recent study, significant amounts of formaldehyde and acetaldehyde were detected at temperatures greater or equal to 215 °C when PG and VG were vaporized by an e-cig, while acrolein was observed when VG was subjected to a temperature in excess of 270 °C (Wang et al., 2017). The heating power of the device is a function of the combination of the coil's resistance value and the voltage applied by the e-cig's battery (i.e., the Joule effect) (Chausse et al., 2015). As such, consumers who use a low-voltage device are able to obtain the same power of a high-voltage e-cig by selecting an appropriate coil.

The aim of the present study was to determine the effect of coil resistance (1.5 Ω and 0.25 Ω coils, to obtain a total wattage of 8 ± 2 W and 40 ± 5 W, respectively) on carbonyl and ROS generation by e-cigs and to evaluate the biological effects of the resulting e-cig vapors on the viability of H1299 human lung adenocarcinoma cells following exposure using an *in vitro* air-liquid interface (ALI) exposure system.

2. Material and Methods

2.1. E-cigarette devices and settings

A commercially available Eleaf Pico e-cig consisting of a 2.5 mL liquid tank made of Pyrex glass and a rechargeable lithium battery (MXJO IMR 18650 3000 mAh 35A 3.7 V High Drain Flat Top Rechargeable Battery) was used for all studies (Figure 1). The voltage value was set at 3.5 V and two different coils (Joyetech™, 1.5 Ω and 0.25 Ω) were used to obtain a total wattage of 8 ± 2 W and 40 ± 5 W, respectively. In order to prevent the confounding effect of coil aging, a new element was used for each experiment.

2.2. E-liquids

Studies to determine carbonyl compounds in e-cig aerosols used a PG/VG base solution (50/50, v/v) (Fumador S.r.l., Milan, Italy) without nicotine (eL-N) and with nicotine (18%; eL+N). A red fruits flavor concentrate (Chemfont S.r.l., Rome, Italy) was then added to a

final concentration of 10% (v/v). For studies examining ROS production and effects cell viability, the e-cig liquid was composed of a PG/VG base (50/50, v/v) (NicVape, USA) without nicotine to which a raspberry flavor concentrate (NicVape, USA) was added at a concentration of 10% (v/v).

2.3. Detection of carbonyl compounds: formaldehyde, acetaldehyde and acrolein

To establish the presence of formaldehyde, acetaldehyde and acrolein, a 30-L propylene box was filled using the following puff profile: puff on 6 s, puff off 5 s; the puffing sequence was repeated twice (Goel et al., 2015; Canistro et al., 2017; Cardenia et al., 2018).

Formaldehyde, acetaldehyde and acrolein were determined by headspace-solid phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC/MS), as reported in our previous study (Canistro et al., 2017) with some modifications. A SPME device having a fused-silica fiber (10-mm length) coated with DVB/CAR/PDMS (50/30 mm thickness), was used. After conditioning at 270 °C for 60 min, the SPME fiber was exposed to the box headspace at room temperature. After a 2-min exposure, the fiber was desorbed at 250 °C for 10 min in the injector of the GC/MS system (Q2010 Plus, Shimadzu, Japan). The sample was injected into a RTX-WAX column (30 m, 0.25 mm i.d., 0.25 µm film thickness, Restek, USA) in split mode (1:20 split ratio). Helium was used as carrier gas with a linear velocity of 36.2 cm/sec. The oven temperature was kept at 35 °C for 10 min, then raised to 240 °C at 30 °C/min. Injector and interface temperatures were set at 250 and 230 °C, respectively. Compounds were recognized by comparing their mass spectra and retention time with those of the corresponding chemical standards. The quantification of formaldehyde, acetaldehyde and acrolein signal was carried out by Single Ion Monitoring (SIM), using 29 *m/z*, 44 *m/z* and 56 *m/z*, respectively. The construction of the calibration curves in this case could not provide reproducible results, due to the difference of vapor pressure of carbonyls when used alone or in presence of other compounds (Liu et al., 2016). Therefore, as suggested in literature (Wang et al., 2017; Geiss et al., 2016), a normalized response factor (Rf) was calculated based on the concentration of carbonyls present in the environment as basal level, according to the following expression:

$$Rf = (A_x - A_y)/A_y$$

where A_x and A_y represent the peak areas of carbonyls detected after and before (basal) the vaping process in the exposure box, respectively.

2.4. Cell-free ROS

The ROS production was estimated using the dye 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), as previously reported by Lerner et al. (Lerner et al., 2015a). To catalyze the reaction between DCFH and ROS, horseradish peroxidase (HRP) was added. Vapor was pulsed into the bubbler (Ace Glass Inc., Vineland, NJ) at room temperature using the following puffing topography: puff on 4 s, puff off 26 s, flow rate 1.5 L/min; this puffing sequence was repeated 15 times, for a total time of exposure of 7.5 min (total number of puffs: 15). The oxidized dichloro-fluorescein (DCF) fluorescence was measured using a Fluroskan Ascent FL spectrofluorometer (Thermo Fisher Scientific Inc., Waltham, MA) at

absorbance/emission maxima of 485 nm/535 nm. H₂O₂ standards were used to calibrate the fluorescence intensity units (FIU) and DCF fluorescence data are expressed as μM of H₂O₂ equivalents. The assay was conducted using both phosphate buffer (PBS) and cell medium as reaction mixture.

2.5. Air-liquid interface cell culture and exposure

H1299 human lung adenocarcinoma cells were purchased from ATCC (Manassas, VA). The cells were cultured in RPMI 1640 basal media supplemented with 10% fetal bovine serum, 1% penicillin and 1% streptomycin at 37°C under a 5% CO₂ atmosphere. Before aerosol exposure, cells were plated in 60-mm dishes and grown to 50–70% confluence. Cells were then exposed to air or e-cig vapor in a modified vacuum desiccator (500 mL volume, SP Scienceware, Warminster, PA) using the following puffing topography: puff on 4 s, puff off 26 s, flow rate 15 L/min; this puffing sequence was repeated 15 times, for a total time of exposure of 7.5 min (total number of puffs: 15). In addition to the analytical needs, this topography was also more representative of the real human use of e-cig and allowed us to reinforce the correlation between our results and the actual health risks for e-cig consumers (Norton et al., 2014; Robinson et al., 2015). This treatment protocol was repeated once after 2 h. Cells were exposed to a total number of 30 puffs.

2.6. Cell viability measurement

The 3-(4,5-dimethylthiazol-3-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Cat#M5655, Sigma Aldrich, St. Louis, MO) was used to assess cell viability 24 h after e-cig vapor exposure. Briefly, 24 h after exposure, H1299 cells were washed twice with PBS and then incubated with MTT (1 mg/mL) in RPMI 1640 medium at 37 °C for 30 min. The medium was then removed and dimethyl sulfoxide (DMSO) was added to solubilize the formazan dye; the absorbance was measured at 550 nm using a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). The viability of cells exposed to e-cig vapor was normalized to the viability of air-exposed cells.

2.7. Statistical analysis

Data referred to as carbonyls compounds are expressed as mean \pm standard deviation (SD) of three independent replicates (n=3) and analyzed by means of one-way ANOVA to evaluate the influence of different conditions tested ($p < 0.05$). Data referred to as DCFH-DA assay in PBS (vapor condensate and air-liquid interface) are expressed as mean \pm standard deviation (SD) of six independent replicates. For DCFH-DA assay in cell medium, results are indicated as relative percentage of the ROS content in control samples arbitrarily set at a value of 100% (mean \pm SD of six independent replicates). Cell viability determined by MTT assay is expressed as percentage variation of viable cells relative to control group arbitrarily set at 100% (mean \pm SD of six independent replicates). One-way ANOVA, followed by Tukey's multiple comparison test, was carried out at a 95% confidence level ($p = 0.05$), to separate means of parameters that were statistically different.

3. Results

3.1. Formaldehyde, acetaldehyde and acrolein levels

In general, eL-N generated higher levels of carbonyls than eL+N. Formaldehyde was the main aldehyde produced in both liquids, followed by acetaldehyde and acrolein; however, the observed concentrations of carbonyls were strictly related to both the composition of liquids and the resistance value. For eL-N, acrolein, acetaldehyde and formaldehyde levels generated by 0.25 Ω coil were three- to seven-fold higher than those generated by the 1.50 Ω coil (Figure 2A). In contrast, the carbonyl content was higher with the 1.50 Ω coil compared to 0.25 Ω coil when eL+N was used (Figure 2B). Figure 2B shows that when the eL+N was used, the 1.50 Ω coil resulted in the highest generation rate of carbonyls, especially formaldehyde, which was significantly ($p<0.05$) higher than acetaldehyde and acrolein; however, the latter was not significantly affected by the resistance value. Since the eL-N led to the highest production of hazardous carbonyls, an eL-N was chosen for subsequent ROS generation and cell viability experiments.

3.2. ROS levels generated by e-cig vapor in PBS

ROS levels generated by e-cigs were determined by collecting vapor in PBS and measured in both the bubbler (vapor condensate) and at the ALI using the exposure chamber described above. Results obtained by analyzing the vapor condensate (Figure 3A) showed more than six-fold higher levels of ROS in samples obtained with the 0.25 Ω coil compared to air control, and about three-fold higher if compared with 1.5 Ω ($p<0.01$). The same trend was found when ROS levels were measured at the ALI (Figure 3B).

3.3. ROS levels generated by e-cig vapor in cell medium

To determine if e-cig vapor produces ROS under cell culture conditions, the DCFH-DA assay was conducted using cell media as solvent under the experimental conditions mentioned previously. In both the bubbler and the ALI exposure chamber, the results followed the same trend as those obtained with PBS. The 0.25 Ω coil produced significantly higher levels of ROS when compared to control in both experimental conditions (+82.9%, $p<0.001$ in vapor condensate; +46.3%, $p<0.05$ in the air-liquid interface) (Figure 3C and 3D). When compared to the control, the 0.25 Ω coil produced increased ROS levels: 143.9% in condensate ($p<0.0002$) and 136.2% in air-liquid interface ($p<0.0001$) (Figure 3C and 3D).

3.4. Cell viability

H1299 cell viability was measured 24 h after exposure in the experimental chamber. Results from the MTT assay (Figure 4A) showed that cell survival was inversely related to the total wattage of the devices. The effect of the 1.5 Ω coil group (10% reduction) had borderline statistical significance ($p=0.058$). Cell viability decreased to 45.8% ($p<0.0001$) when cells were exposed to the vapor generated by using the 0.25 Ω coil. Cell viability was observed to be inversely correlated to ROS production in cell medium revealed in the exposure chamber (Figure 4B).

4. Discussion

E-cigs are composed of several components which, in many cases, can be modulated or modified according to a consumer's preferences. Among the modifiable components, the coil that is present in the atomizer is the element that, in combination with the applied voltage, is responsible for heating the liquid, which is generally composed of variable percentages of PG, VG, flavors and nicotine. It is well-established that thermal decomposition of the PG-VG mixture leads to the formation of toxic and carcinogenic carbonyls, such as formaldehyde, acetaldehyde and acrolein (Paschke et al., 2014; Uchiyama et al., 2013). Several studies have also reported that e-cig vapor contains relatively high levels of free radical species (Lerner et al., 2015a; Lerner et al., 2015b; Sussan et al., 2015; Pryor et al., 1997), which are known to be important causal factors in many tobacco related diseases and disorders, such as cardiovascular diseases, COPD and cancer (11Messner et al., 2014; Dekhuijzen, 2004; MacNee and Rahman, 2001).

In the present study, we investigated the role of an e-cig's heating coil resistance with respect to the generation of carbonyls and ROS. Here, we report for the first time that the levels of selected hazardous carbonyls and ROS increases as coil resistance is reduced. The presence or absence of nicotine was also observed to affect the production of carbonyls with eL-N producing higher levels of carbonyls than eL+N. As reported by Kosmider et al. (2014), the composition of e-cig significantly affects the composition of the released carbonyl compounds. In general, the composition of the volatile fraction is strictly linked to the thermodynamic equilibrium between the vapor and condensed (liquid) phases; for this reason, when the number and/or the concentration of e-cig liquid ingredients decreases, the competition between molecules in the headspace decreases as well, thus leading to an increase and accumulation of some volatile compounds in the headspace, such as the revealed carbonyl compounds that are characterized by a high vapor pressure. Our results are consistent with previous studies demonstrating that the level of carbonyl compounds in e-cig vapors is affected by presence of nicotine and battery voltage (Kosmider et al., 2014). The latter is a relevant issue considering that e-cigs have become popular during the past decade (Korzun et al., 2019) for having the capacity of modulating the nicotine content of the liquid. Human exposure to these low molecular weight carbonyls represents a risk factor for occurrence of neoplastic diseases. In particular, formaldehyde and acetaldehyde are classified as Group 1 and Group 2B carcinogens, respectively, by the International Agency for Research on Cancer (IARC, 2012). Acrolein is listed as a hazardous air pollutant by the United States Environmental Protection Agency (U.S. EPA, 2003). The presence of higher carbonyls levels in eL-N liquid overturns the idea that the absence of nicotine makes the liquid safer. There is no doubt that nicotine is the molecule responsible for addiction and its employment in e-cig is considered a gateway towards the consumption of tobacco cigarette. However, the toxic carbonyls detected in this study represent a risk factor for the onset and development of neoplastic and chronic diseases (Primack et al., 2015; Soneji et al., 2018). This is of particular concern considering that, in many Countries, the nicotine-free e-cig can be sold to adolescent younger than 18 years old.

The generation of carbonyls is related to the formation of radicals (e.g., hydroxyl radicals), which are responsible for the oxidation and fragmentation of glycols (Geiss et al., 2016). For

this reason, the ROS levels were measured in both vapor and cell medium using the DCFH-DA assay in a cell-free system. Although the exposure profile was different from the one used for detecting the selected carbonyl compounds, the results exhibited the same trend. The reduction of the coil resistance, and thus the increase of the heating and the total wattage of the device, played an important role in terms of ROS formation. Our results appear to confirm the hypothesis of Lerner et al. (Lerner et al., 2015a) who reported that one of the possible sources of ROS could be the heating element. We examined the biological implications of the ROS and carbonyls by examining the effect of e-cig vapor on the viability of H1299 human lung adenocarcinoma cells. We found that vapor from eL-N significantly reduced cell viability and that these effects were directly related to the levels of ROS produced.

High levels of ROS represent a risk factor for the onset of several diseases and pathological conditions, including cancer and inflammation (Waris and Ahsan, 2006). ROS are able to create DNA strand breaks, cross-links, and can cause modification to the purine, pyrimidine and deoxyribose components of DNA (Halliwell and Gutteridge, 2015). In an *in-vivo* study, e-cig aerosol caused accumulation of 8-hydroxy-deoxyguanosine (8-OH-dG) which can lead to mutations, with a significant correlation with ROS content, thus probably resulting in inflammatory response and neoplastic development (Canistro et al., 2017).

Others have previously examined the impact of e-cig vapor and e-cig extract on the morphology and viability of lung cells using both air-liquid interface or direct exposure to e-cig extract (Lerner et al., 2015b; Cervellati et al., 2014; Higham et al., 2018). *Ex vivo* treatment of bronco-epithelial cells from both patients with COPD and healthy subjects with e-cig extracts increased some inflammatory responses including altered cytokine and chemokine production (Higham et al., 2018).

In support of these data, epidemiological studies have indicated an association between e-cig use and respiratory disorders, especially asthma, bronchitis and COPD (Choi and Bernat, 2018; McConnell et al., 2017; Schweitzer et al., 2017; Wills et al., 2019). Altogether, these findings confirm the pivotal role of oxidative stress induced by e-cig use in the onset and development of respiratory diseases in healthy subjects or in the transition of initial symptomatology in chronic disorders.

5. Conclusion

In conclusion, the technology of newer generation e-cigs allow users to easily switch among heating elements in order to generate more or less aerosols and/or to intensify or reduce the flavor intensity of e-liquids. The ability to manipulate these parameters, together with the option that consumers have to select different e-liquid flavors and nicotine content, allows users to use e-cigs without any indication on its potential risks. Since the device and liquids used in this study are commercially-available, we believe that the exposure conditions, and consequently the generated carbonyls and ROS levels, are relevant to public health. Based on the results of this present study, the use of lower resistance coils coupled with an intermediate voltage setting (i.e., 3.5 V) can potentially represent a considerable health concern. Our results indicate how the generation of thermal degradation by-products

depends not only on the applied voltage but also on resistance. Our study demonstrates the need for e-cig consumers to be cautious when assuming that low-voltages may be synonymous with “safer” devices (Thomson and Lewis, 2015), and in a broader sense, reiterates that after more than a decade of research, a “safe level” of exposure from e-cig aerosols cannot yet be established.

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Abbreviations

ROS	reactive oxygen species
COPD	chronic obstructive pulmonary disease
PG	propylene glycol
VG	vegetable glycerol
DCFH-DA	2',7'-dichloro-dihydro-fluorescein diacetate
HRP	horseradish peroxidase
DCF	dichloro-fluorescein
H₂O₂	hydrogen peroxide
MTT	3-(4,5-Dimethylthiazol-3-yl)-2,5-diphenyl Tetrazolium Bromide
FIU	fluorescence intensity units
IARC	International Agency for Research on Cancer
U.S. EPA	United States Environmental Protection Agency
HS-SPME	headspace-solid phase microextraction
GC/MS	gas chromatography/mass spectrometry
R_f	response factor
eL-N	liquid without nicotine
eL+N	liquid with nicotine
ALI	air-liquid interface

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Highlights

- Nicotine-free liquid led to higher levels of carbonyl compounds in vapor.
- Low resistance coil is associated with higher levels of carbonyls and ROS.
- 0.25 Ω coil vapor was more toxic to lung cancer cells than 1.5 Ω coil vapor.
- Cell viability in each exposed group inversely correlates vs ROS generation.

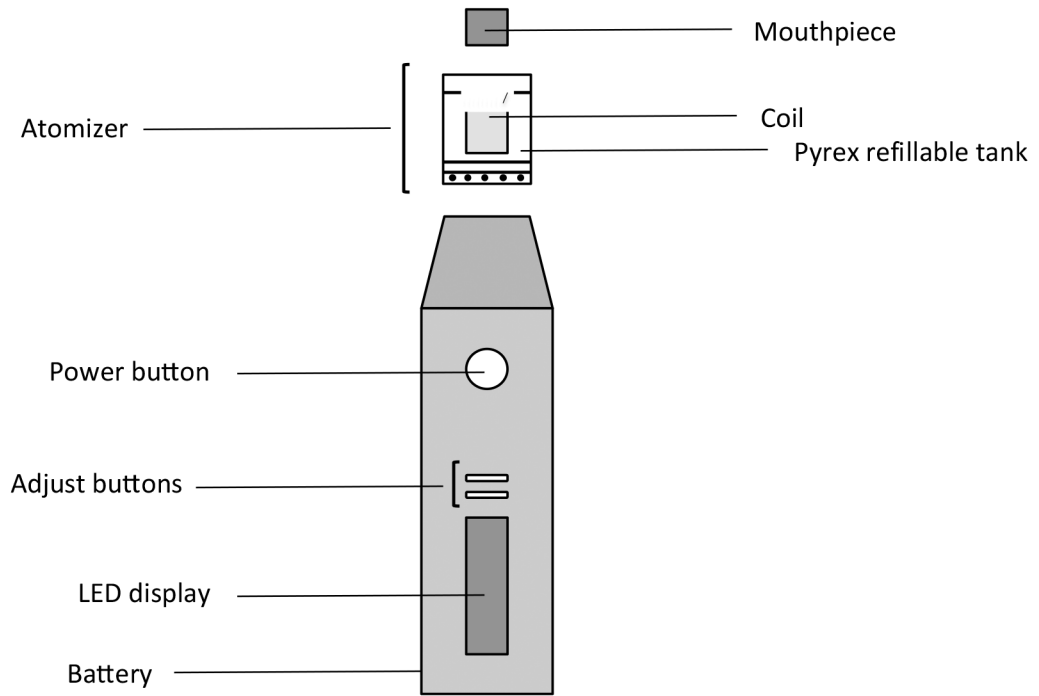


Figure 1.
Schematic representation of the e-cig device used for the study.

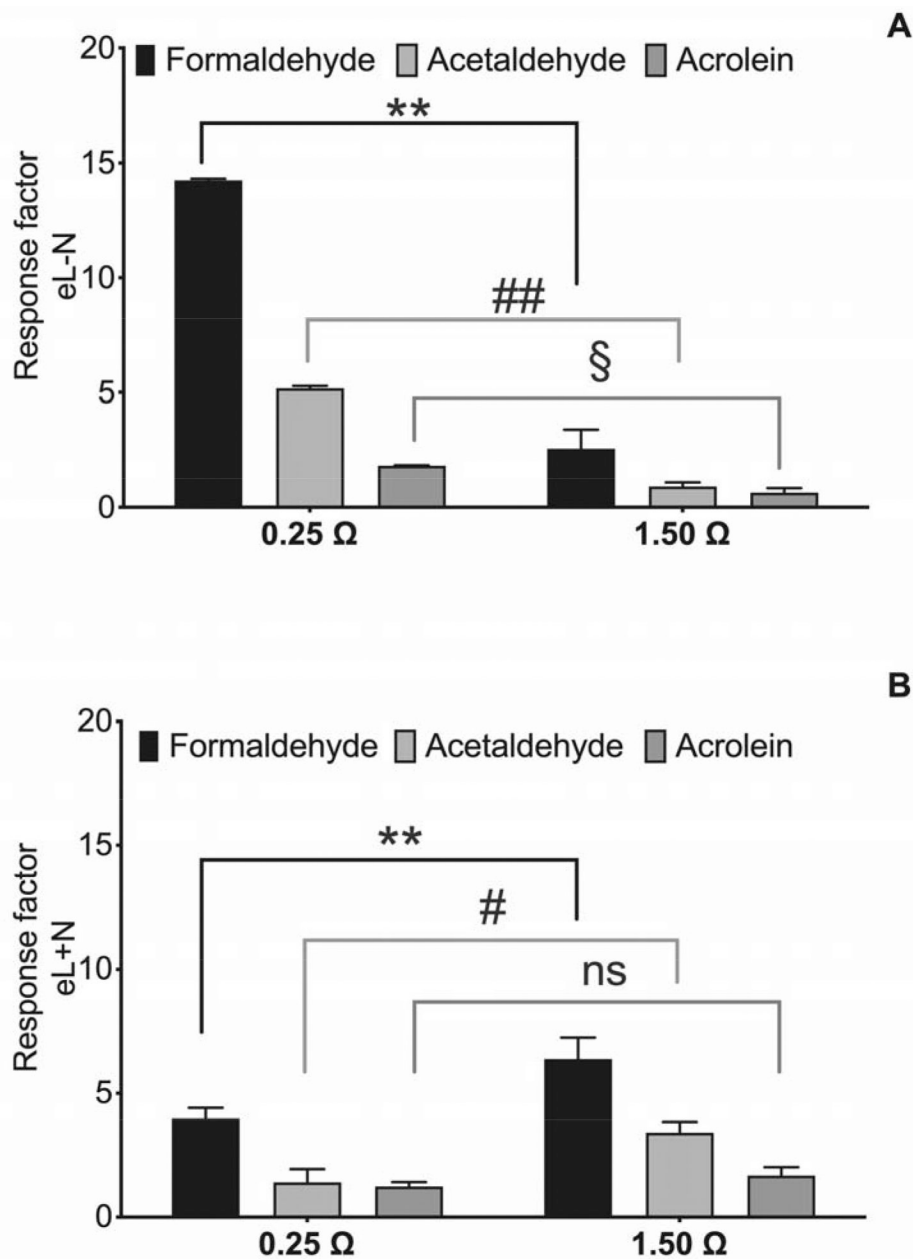


Figure 2. Effects of resistance value (0.25 Ω and 1.5 Ω) on formaldehyde, acetaldehyde and acrolein levels in vapors released by eL-N (without nicotine) (A) and eL+N (with nicotine) (B). Data represent mean ± SD of three independent replicates. Different letters (a-b) for each aldehyde denote statistically different means (Tukey’s test; $p < 0.05$) related to the resistance values; different letters (x-z) for each resistance value denote statistically different means (Tukey’s test; $p < 0.05$) related to carbonyls.

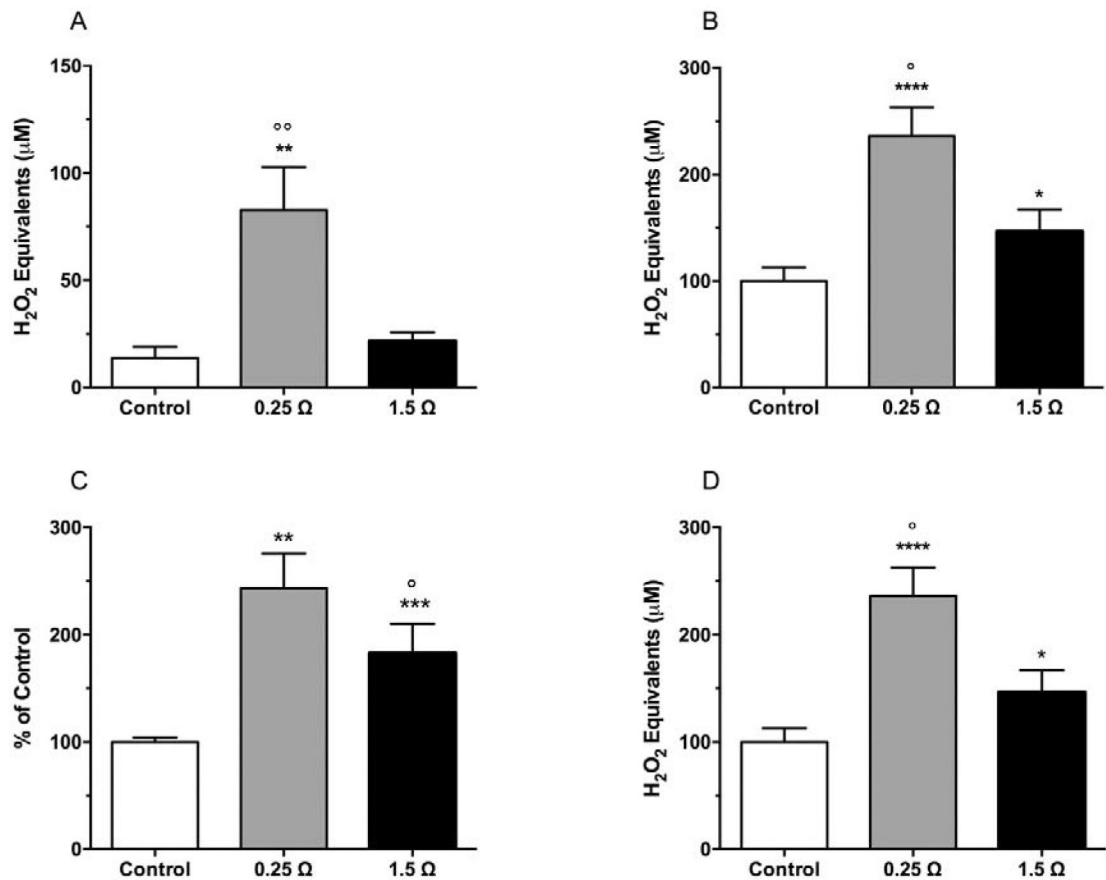


Figure 3.

Effect of resistance values on ROS levels in e-cig vapor. Fresh air control and e-cig vapor were pulsed through DCFH-HRP ROS indicator solution in (A, B) phosphate buffer and (C, D) cell culture media in (A, C) vapor condensate and (B, D) the ALI during a 7.50 min exposure. Data represent mean \pm SD of six independent replicates.

** $p < 0.01$; *** $p < 0.0002$ significant results between 1.5 Ω/0.25 Ω groups and control group using one-way ANOVA (Tukey's multiple comparison test).

° $p < 0.05$; °° $p < 0.01$ significant results between 0.25 Ω and 1.5 Ω group using one-way ANOVA (Tukey's multiple comparison test).

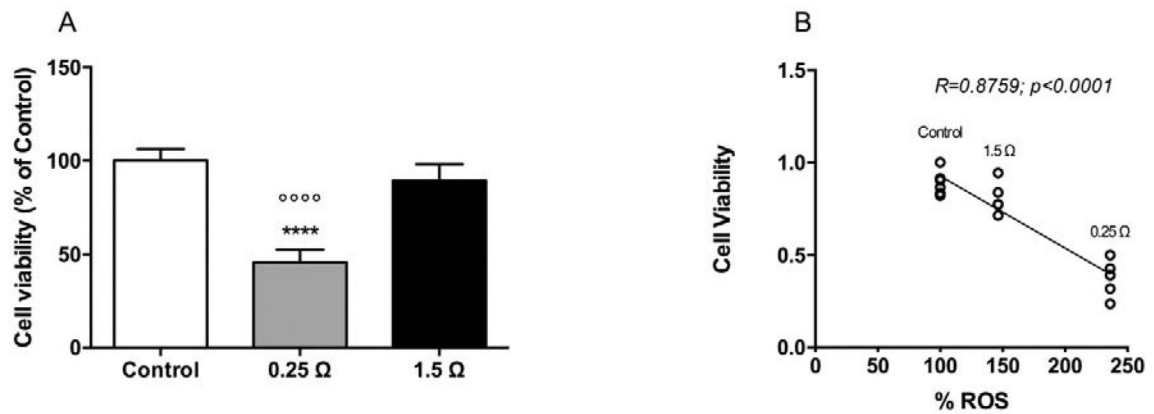


Figure 4.

Relationship between cell viability, coil resistance and ROS generation. (A) Effect of vapor generated from e-cigs equipped with 1.5 Ω and 0.25 Ω coils on viability of H1299 cells by MTT assay. Data are expressed as percentage relative to viable cells in control arbitrarily set at a value of 100%; Data represent mean \pm SD of six independent replicates. ** $p < 0.01$; *** $p < 0.0002$ significant results between 1.5 Ω/0.25 Ω groups and control group using one-way ANOVA (Tukey's multiple comparison test).

$^{\circ}p < 0.05$; $^{\circ\circ}p < 0.01$ significant results between 0.25 Ω and 1.5 Ω group using one-way ANOVA (Tukey's multiple comparison test). (B) Cell viability in each group inversely correlates vs ROS generation ($R = 0.8759$; $p < 0.0001$).