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MINIREVIEW

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Immunological and pathological effects of electronic cigarettes

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Abstract

Electronic cigarettes (E-cigarettes) are considered a preferable alternative to conventional cigarettes due to the lack of combustion and the absence of tobacco-specific toxicants. E-cigarettes have rapidly gained in popularity in recent years amongst both existing smokers and previous non-smokers. However, a growing literature demonstrates that E-cigarettes are not as safe as generally believed. Here, we discuss the immunological, and other, deleterious effects of E-cigarettes on a variety of cell types and host defence mechanisms in humans and in murine models. We review not only the effects of complete E-cigarette liquids, but also each of the main components—nicotine, humectants and flavourings. This MiniReview thus highlights the possible role of E-cigarettes in the pathogenesis of disease and raises awareness of the potential harm that E-cigarettes may cause.

KEYWORDS

E-cigarette, flavourings, humectants, immunological effects, nicotine, tobacco

1 | INTRODUCTION

Electronic cigarettes (E-cigarettes) are hand-held electronic devices that generate vapours or aerosols from E-cigarette liquid (E-liquid) without combustion. The main components of E-liquid are humectants (ie, propylene glycol, vegetable glycerol), nicotine and flavourings. The main compartments of an E-cigarette comprise a mouthpiece, a cartridge, a heating element/atomizer and a battery (Figure 1). E-liquid is stored in the cartridge and then supplied to the atomizer which contains a small coil of electrically resistant wire that heats up when the battery is switched on.

Electronic cigarettes have gained rapidly in popularity around the world in recent years; sales of E-cigarette may even surpass conventional cigarettes by 2023.[1] Since E-cigarettes were invented in 2003, overall usage increased from 3.3% to 8.5% between 2010 and 2013 amongst adults in the USA[2] and the experience of E-cigarette usage rose from 4.6% to 8.2% in 2014 amongst young people aged 11-18 in the UK.[3] In addition, a rapid rise in E-cigarette use has occurred not only amongst current smokers, but also non-smokers who may therefore develop a smoking habit and/or nicotine addiction.[4]

Public Health England promoted the use of E-cigarettes as smoking cessation aids, which could be contributing to at least 20 000 successful new quits per year, and many E-cigarette smokers believe this to be a benefit of E-cigarettes.[5] Long-term E-cigarette use can assist those tobacco smokers who attempt to quit smoking to reduce cigarette smoke (CS) consumption, without severe withdrawal symptoms.[6] The increased prevalence of E-cigarette usage is positively correlated to success in quitting cigarette smoking.[7] Evidence also shows that E-cigarette use helps smokers to commit to smoking cessation, thus leading to fewer smoking-related diseases.[8,9]

In addition to promoting abstinence from smoking, E-cigarettes may help those who cannot totally quit smoking to reduce their exposure to tobacco-specific toxicants. Manufacturers also promote E-cigarettes as a safe alternative to conventional cigarettes due to lower levels of carcinogens and toxicants in vapours.[10] These recommendations were

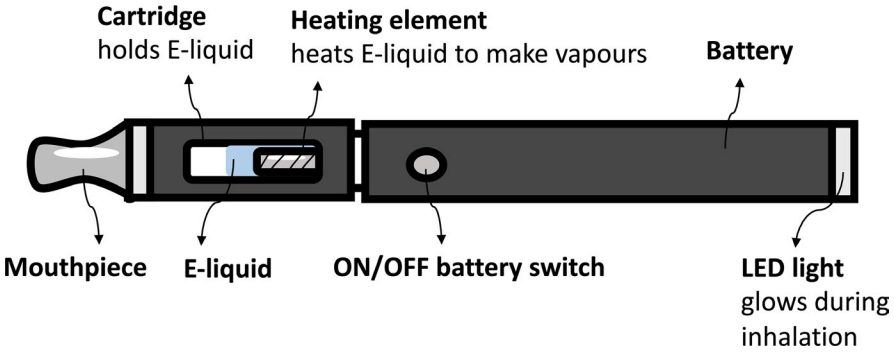

FIGURE 1 Structure of a second-generation electronic cigarette

supported by The Royal College of Physicians in the UK. The most common reasons given for the use of E-cigarettes amongst pregnant women were the perception of less harm than CS and help with smoking cessation. An online survey demonstrated that the majority of the respondents believed that E-cigarettes are less harmful than tobacco smoking and that they have few side effects, with less coughing and better breathing.[11] Furthermore, E-cigarette users had positive attitudes towards E-cigarettes as they regarded the ingredients to be less harmful than CS, although this judgement was based on incomplete information and knowledge.[12]

The World Health Organization (WHO) does not recommend the use of E-cigarettes for never smokers, children and pregnant women[13] and strongly advises consumers not to use E-cigarettes until a reputable regulatory body has found them to be safe.[14] The sale of E-cigarettes is already banned in 25 of 68 countries that regulate them,[15] and the US Food and Drug Administration (FDA) has proposed a range of regulatory tools to prohibit flavoured E-cigarettes recently.[16] Moreover, despite the positive claims of manufacturers and the perceptions of the public, the use of E-cigarettes remains controversial, and a growing literature provides evidence for deleterious effects of E-cigarettes. Here, we discuss the immunological, and other, effects of E-cigarette on a variety of cell types in humans and in murine models, discussing both complete E-liquids and their main constituents (Figure 2). This MiniReview might help the public to consider whether to use E-cigarettes based on scientific evidence.

2 | EFFECTS OF E-CIGARETTES IN HUMANS

2.1 | Benefits of E-cigarette use

Users of conventional cigarettes who switched to E-cigarettes reported reduced nicotine withdrawal symptoms with less cigarette craving and improved chest tightness and other health effects over the course of 2 weeks.[17] Substitution of cigarettes by nicotine-containing E-cigarettes also reduced the exposure to carcinogens and toxicants commonly found in CS, assessed by urine biomarkers for tobacco-specific nitrosamine, volatile organic compounds and polycyclic aromatic hydrocarbons. A one-year, randomized, controlled trial of CS smokers, who completely stopped smoking or switched to E-cigarette use, showed progressive improvements in respiratory symptoms, particularly coughing and shortness of breath.[18] A 1-year prospective, randomized control study found restoration of "fractional nitric oxide concentration in exhaled breath" (FeNO) and reduction in "exhaled carbon dioxide" (eCO) in CS users who completely stopped cigarette smoking after switching to E-cigarettes, along with improvements in symptom scores.[19] Nitric oxide is involved in antimicrobial activity against lung pathogens and has antitumour effects, whereas eCO is a biomarker of smoking that reflects airway inflammation; thus, normalization of FeNO and eCO might support the use of E-cigarettes to reverse the harmful effects of CS in the lungs. A prospective 3.5-year observational study of nine E-cigarette users who never smoked conventional cigarettes found E-cigarette use did not alter healthy outcome including blood pressure, heart rate, body-weight, lung function, respiratory symptoms, exhaled breath nitric oxide (eNO), exhaled carbon monoxide (eCO) or high-resolution computed tomography of the lungs.[20]

In addition to helping to reduce harmful effects in smokers with normal lung function, E-cigarette use may also benefit patients with respiratory and systemic diseases. COPD patients who switched to the use of E-cigarettes reduced their CS consumption, and more than half stopped cigarette smoking completely during a 12-month follow-up.[21] They also had significant reduction in annual COPD exacerbation, rate of FEV1 decline and improved symptoms and ability to perform daily physical activities. Similarly, a marked attenuation in CS consumption was found in asthma patients who switched to E-cigarettes, with better asthma control, lung function and lower airway hyperresponsiveness over a 2-year period.[22] Regular E-cigarette use could help reduce CS consumption in arterial hypertensive patients, further showing good blood pressure control and disease severity after 12 months' follow-up.[23]

2.2 | Effects of E-cigarettes on the oral cavity

Nicotine has been demonstrated to alter the cytoskeleton[24] and to induce extracellular matrix remodelling[25] in gingival

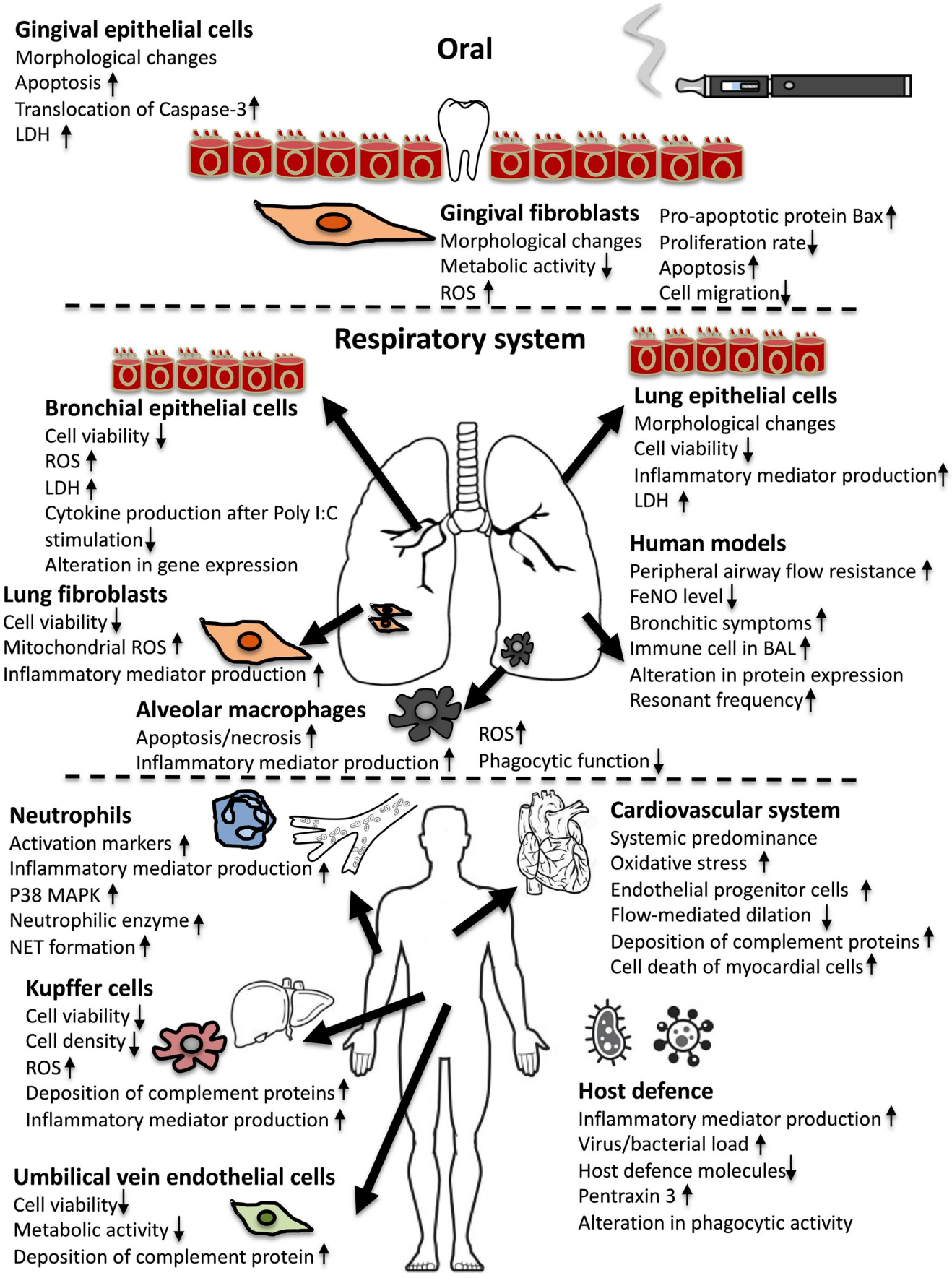

FIGURE 2 Effects of electronic cigarettes in humans

TABLE 1 The effects of CS and E-cigarette on oral cavity

Effects	CS	E-cigarette
Gingival fibroblast		
Metabolic activity	Decrease[26]	Decrease[27]
Alteration in morphology	Yes[28]	Yes[28]
Proliferation rate	Decrease[28]	Decrease[28]
Apoptosis	Increase[28,30]	Increase[27,28]
Cell migration	Decrease[28]	Decrease[28]
Wound closure rate	Decrease[28]	Decrease[28]
ROS production	Increase[29]	Increase[27]
Pro-apoptotic protein Bax expression	Increase[30]	Increase[27]
Gingival epithelial cell		
Apoptosis	Increase[31]	Increase[32]
Caspase-3 activity	Increase[31]	Increase[32]
Alteration in morphology	Yes[31] (disorganization of epithelium)	Yes[32]
Lactate dehydrogenase (LDH) activity	N/A	Increase[32]

CS: cigarette smoke; E-cigarette: electronic cigarette.

fibroblasts—a cell type that is directly exposed to CS constituents during smoking (Table 1). CS inhibited metabolic ability of human gingival fibroblasts time-dependently, as assessed by MTT assay.[26] Exposure of human gingival fibroblasts to E-liquid, with and without nicotine, reduced their metabolic activity in a dose- and time-dependent manner.[27] E-liquid with nicotine concentrations of greater than 1 mg/mL showed pronounced toxicity that was further exacerbated by vaporizing the fluid. Consistent with the study treating human gingival fibroblasts, both CSE and E-cigarette vapour extract (ECVE) altered morphology, proliferation rate, apoptosis, migration and wound closure.[28] Of note, CSE and ECVE with nicotine exert greater magnitude of damage than those without nicotine. It has been reported that CS triggered the production of reactive oxygen species (ROS) from human gingival fibroblasts.[29] Similarly, ROS production was increased significantly after 24-hour exposure of gingival fibroblasts to either nicotine-containing or nicotine-free fluids and vapours, indicating that nicotine was not the only inducer of ROS production.[27] Levels of the pro-apoptotic protein Bax, which is involved in triggering ROS production, is up-regulated by CSE.[30] Bax expression was also elevated after exposure to nicotine-containing and nicotine-free E-liquids and vapours for 24 hours, followed by cell apoptosis after 48 hours.[27]

The epithelium forms the first line of defence against toxic substances and periodontal pathogens. CS induced human gingival epithelial cell apoptosis, which was confirmed by up-regulated expression of Bax and caspase-3 activity. CS also inhibited gingival epithelial cell migration, which reduced the percentage of healing in a scratch assay. In addition, CS reduced the numbers of epithelial cell layers, resulting in epithelial disorganization.[31] Exposure of primary human gingival epithelial cells to ECV raised levels of apoptosis associated with increased caspase-3 activity accompanied by morphological changes and increased lactate dehydrogenase (LDH) activity.[32]

2.3 | Effects of E-cigarettes on the respiratory system

After the oral cavity, airway epithelium is the next site of contact with inhaled substances and serves as an active contributor in defence of the respiratory system (Table 2). Damage to, or alteration of, airway epithelial cells by inhaled particles can increase the permeability of the epithelium, leading to inflammatory cell influx into the airways. These infiltrating cells contribute to persistent inflammation and the secretion of mediators that cause lung tissue destruction, including defensins, elastase and matrix metalloproteinases (MMPs). Epithelial cells themselves, through production of these mediators, can also participate in the destructive processes.

Several studies have compared the effects of the constituents of E-cigarettes and tobacco on cells of the human respiratory tract, using either primary cells or cell lines. In one study using primary human bronchial epithelial cells (HBECs) obtained at surgery, exposure of the cultured cells to either ECV or CS at an air-liquid interface caused reduced cell viability and increased oxidative stress.[33] These effects were seen using ECV with or without nicotine; indeed, similar deleterious effects were induced by the humectants alone (ie, propylene glycol and glycerol) that are used in the E-liquids. However, the increase in cell death and oxidative stress of the HBECs was much more pronounced when exposed to CS than to ECV. In contrast, another study observed CSE induced apoptosis and necrosis in HBECs assessed by increasing caspase 3/7 activity and reducing viable cell protease activities, and disrupted cellular redox haemostasis characterized by reduction in glutathione ratio and increase in intracellular ROS. However, nicotine-containing ECVE did not elicit those effects.[34]

Another study also investigated the exposure of primary HBECs to ECV and CS at an air-liquid interface, using gene-expression profiling as the read-out.[35] Genes up-regulated by both CS and ECV were related to apoptosis, xenobiotic stress and oxidative stress. In addition, CS and ECV down-regulated the expression of genes associated with the assembly and movement of cilia, which might cause a reduction of ciliated cells in airway epithelium and compromise mucociliary clearance function—this could contribute to chronic inflammation in the airways. ECV containing nicotine had more pronounced effects than those without nicotine. This study showed that ECV could induce similar effects to CS; again, however, the effects of CS were more pronounced.

TABLE 2 The effects of CS and E-cigarette on respiratory system

Effects	CS	E-cigarette
Bronchial epithelial cells		
Cell viability	Decrease[33]	Decrease[33]
Oxidative stress	Increase[33,34]	Increase[33] No[34]
Apoptosis	Increase[34]	No[34]
Necrosis	Increase[34]	No[34]
Caspase 3/7 activity	Increase	No[34]
Glutathione ratio	Increase[34]	No[34]
Alteration in gene expression	Yes[35]	Yes[35]
LDH release	No[38]	Increase[38]
Cytokine production (ie IL-6, CXCL10 and CCL5) after Poly I:C stimulation	Decrease[38]	Decrease[38]
Lung epithelial cells		
Morphological changes	Yes[36]	Yes[36]
LDH release	Increase[36]	Increase[36]
Cell viability	Decrease[36,37]	Decrease[36] No[37]
Oxidative stress	Increase[37]	No[37]
Inflammatory mediator production (ie IL-8, MCP-1 and GRO-α)	Decrease[37]	No[37]
IL-6 production	Increase[37]	Increase[37]
Surfactant		
Alteration in microstructure	Yes[39]	Yes[39]
Surface tension	Yes[39]	Yes[39]
Lung fibroblasts		
Cell viability	Decrease[40]	Decrease[40]
Inflammatory mediator production (ie IL-6 and IL-8)	Increase[40]	Increase[40,41]
Mitochondrial ROS production	Increase[42]	Increase[41]
Alveolar macrophage		
Apoptosis/necrosis	Increase[44]	Increase[43]
Inflammatory mediator production	Increase[45-47]	Increase[43]
ROS production	Increase[48]	Increase[43]
Phagocytic function	Decrease[46]	Decrease[43]
Human models		
Peripheral airway flow resistance	Increase[49]	Increase[51]
FeNO level	Decrease[49]	Decrease[51,55]
Bronchitic symptoms	Increase[50]	Increase[52]
Alteration in transcriptomes/ protein expression	Yes[54]	Yes[53,54]
Resonant frequency	N/A	Increase[55]

CS: cigarette smoke; E-cigarette: electronic cigarette; FeNO: fractional exhaled nitric oxide; IL: interleukin; LDH: lactate dehydrogenase; ROS: reactive oxygen species.

Human cell lines derived from lung epithelium (A549) and skin keratinocytes (HaCaT) have also been used to study the effects of CS, ECV containing nicotine and/or balsamic flavouring, and ECV humectants alone.[36] CS and, to a lesser extent, ECV containing balsamic flavouring or nicotine triggered morphological changes, elevated LDH release and reduced viability in both cell types. The influence of flavouring was greater than nicotine and the ECV humectants alone did not have these effects. However, exposure to the humectants did induce release of multiple cytokines by the lung epithelial cells. This paper concluded that ECV was less cytotoxic than CS and that the deleterious effects of E-cigarettes were more related to the flavourings than to nicotine. A study examining the effects of CS and ECV on the HBEC cell line BEAS-2B also concluded that the deleterious effects of E-cigarettes were less severe than those of CS.[37]

Electronic cigarette vapour extract also significantly increased LDH release from bronchial epithelial cells (BECs) in both healthy and COPD patients and induced production of cytokines, including interleukin (IL)-6 and IL-8. Moreover, both CS and ECVE exposure to BECs from COPD patients showed much lower IL-6, CXCL10 and CCL5 production than those from healthy donors after Poly I:C stimulation. Due to the role in antiviral responses by recruiting and activating lymphocytes, suppression of CXCL10 and CCL5 could reduce antiviral activity in bronchial epithelium, leading to elevated susceptibility to infection.[38]

Surfactant helps to reduce surface tension of alveolar fluid; therefore, less energy is required to inflate the lungs and prevent alveolar collapse. High surface tension is a known characteristic of asthma, pneumonia and COPD. Both CS and ECVs affected surfactant microstructure analysed by Infasurf®, a calf lung surfactant extract used as a lung surfactant model. However, surface tension was increased by CS but not ECVs. Interestingly, tar was the only component in CS able to increase surface tension, suggesting E-cigarette did not alter surface tension due to lack of tar.[39]

Experiments with the human lung fibroblast line, HFL-1, showed that these cells, like lung epithelial cells, are sensitive to ECV. Again, however, the cells were more sensitive to CS than to ECV in the sense that CS, but not ECV, reduced the fibroblast viability in relatively large culture volumes, whereas ECV did reduce the viability only of smaller cultures.[40] Certain flavours of ECV, as well as CS, also induced IL-6 and IL-8 production.[40,41] Acute increase in mitochondrial ROS was also observed in the human lung fibroblast cell line treated with nicotine-containing ECVs,[41] consistent with findings using CS.[42]

Electronic cigarette vapour condensate (ECVC) caused more reduced viability of alveolar macrophages than E-liquid.[43] ECVC also induced apoptosis, necrosis, ROS production and pro-inflammatory cytokines (ie IL-6, TNF-α, IL-8), chemokine MCP-1 and proteolytic enzyme MMP-9

production but decrease phagocytic function of alveolar macrophages, and these impacts were ameliorated by antioxidant, suggesting the role of oxidative stress in E-cigarette. Similar to ECV, CS also has been reported to trigger the apoptosis,[44] inflammatory mediators[45-47] and ROS[48] but attenuate phagocytosis of alveolar macrophages.

Cigarette smoke is known to have deleterious effects on the respiratory system in humans[49,50] and studies in human subjects also raised concerns over the safety of E-cigarette use. Healthy smokers, who used E-cigarettes for 5 minutes, had increased levels of peripheral airway flow resistance and oxidative stress illustrated by lower fractional exhaled nitric oxide (FeNO), demonstrating the rapid side effects of E-cigarettes.[51] Adolescents using E-cigarettes for 12 months showed a twofold increase in rates of chronic bronchitic symptoms, such as chronic cough, phlegm or bronchitis.[52] Acute E-cigarette use (regardless of nicotine content) by never-smokers altered transcriptomes in small airway epithelium including downstream targets of p53 signalling related to apoptosis, cell cycle arrest and DNA damage.[53] Moreover, E-cigarette use changed transcriptomes in alveolar macrophages, thereby inhibiting their migration and phagocytic ability but increased susceptibility of E-cigarette users to infection.[53] Both chronic CS and E-cigarette users had more erythematous and irritable airway mucosa, and there were approximately 200 proteins altered (78 proteins altered in both groups and 113 specifically altered in vapers) in bronchial epithelium, including mucin MUC5AC that facilitates mucus secretion. Of note, some proteins up-regulated in vapers like MUC5AC, CYP1B (generates covalent adducts to damage DNA) and STIM1 (controls Ca^{2+} homeostasis) were also elevated by aerosolized PG/VG in HBECs in vitro.[54] Aside from direct use, a 30-minute passive exposure to E-cigarette vapour in healthy non-smokers caused small airway irritation and inflammation detected by increased resonant frequency and reduced FeNO, raising the possibility of potential risk to humans exposed to ECV in public places and environments.[55]

Overall, the studies considered here indicate that ECV is less deleterious to the respiratory system than CS, but still has significant toxic and inflammatory effects whose long-term consequences are currently unknown.

2.4 | Effects of E-cigarettes on other cells and tissues systemically

Inhaled substances can be absorbed into the bloodstream and distributed systemically (Table 3). It is therefore important to consider possible effects of ECV constituents on other cells and tissues in addition to effects on cells of the respiratory tract.

Neutrophils are an important component of innate immunity that act rapidly against invading pathogens by phagocytosis, degranulation and release of neutrophil extracellular traps. They are abundant in the circulation and migrate to sites of infection in response to chemokines and other chemo-attractants. Neutrophils also release cytokines which can recruit and activate other cells during inflammation. Neutrophils treated with ECV extract for up to 6 hours showed increased expression of the neutrophil activation markers CD11b and CD66b[56]; this is in contrast with a report that CD11b was up-regulated in circulating neutrophils from tobacco smokers only with COPD, not from tobacco smokers with normal lung function.[57] Also, the levels of neutrophil elastase, MMP-9 and IL-8 showed similar changes, accompanied by p38 MAPK activation without a reduction in neutrophil viability. CD66b cross-linkage between neutrophils was increased by ECV extract, which

TABLE 3 The effects of CS and E-cigarette on other cells and tissue systemically

Effect	CS	E-cigarette
Neutrophils		
Activation marker (ie CD11b and CD66b)	No[57]	Increase[56]
Inflammatory mediator production	Increase[56]	Increase[56]
p38 MAPK activation	N/A	Increase[56]
Neutrophilic enzyme	Increase[58]	Increase[58]
NET formation	Increase[58]	Increase[58]
Cell number	Increase[58]	No[58]
Cardiovascular system		
Sympathetic predominance	N/A	Yes[59]
Oxidative stress	Increase[61]	Increase[59,61]
Endothelial progenitor cells	Increase[60]	Increase[60]
Flow-mediated dilation	Decrease[61]	Decrease[61]
Antioxidant vitamin E	Decrease[61]	Decrease[61]
Umbilical vein endothelial cells		
Cell viability	Decrease[62]	Decrease[62]
Metabolic activity	Decrease[62]	Decrease[62]
Complement deposition	Increase[62]	Increase[62]
Migration	Decrease[63]	No[63]
Myocardial cells		
Cell death	Increase[64]	Increase[64]
Morphological changes	Yes[64]	No[64]
Kupffer cells		
Cell viability	Decrease[65]	Decrease[65]
Cell density	Decrease[65]	Decrease[65]
Complement deposition	Increase[65]	Increase[65]
Oxidative stress	Increase[65]	Increase[65]
Inflammatory mediator production	Increase[65]	Increase[65]

CS: cigarette smoke; E-cigarette: electronic cigarette.

might cause the secretion of IL-8. Neutrophil granulocytes possess two mechanisms for antimicrobial activity: degranulation of stored mediators and release of NETs. The levels of neutrophilic enzymes associated with chronic lung diseases, such as neutrophil elastase, myeloperoxidase and matrix metalloproteinase-9 and NET formation, were elevated in sputum derived from both CS and E-cigarette users, with increasing neutrophil count by CS only.[58] In addition, E-cigarettes suppressed antimicrobial activity and viscoelastic properties of the mucus barrier, as demonstrated by decreasing innate defence proteins secreted by the airway epithelium. Interestingly, these proteins were elevated in tobacco smokers. Increased total mucin concentration, an important parameter for failed mucus transport in muco-obstructive disease and COPD, was shown in both CS and E-cigarette users. These results suggested ECV might affect neutrophilic response and defence proteins in lungs in a similar way to that seen in CS users.

Habitual E-cigarette users had a shift in cardiac autonomic balance towards sympathetic predominance and higher systemic oxidative stress levels, which are known risks for cardiovascular disease (CVD).[59] The levels of endothelial progenitor cells, the biomarker for endothelial function and risk marker for CVD based on their function in maintenance, differentiation and regeneration of endothelial cells following vascular injury or necrosis, were elevated in participants exposed to ECV acutely, similar to exposure to CS.[60] Flow-mediated dilation, the other marker for endothelial function and CVD, was decreased after CS and E-cigarette use.[61] Markers of oxidative stress were elevated, but antioxidant vitamin E was suppressed by both acute CS and E-cigarette exposure.[61] Viability and metabolic activity of human umbilical vein endothelial cells (HUVECs) was inhibited by CSE and nicotine-containing ECVE but not pure nicotine.[62] Furthermore, the deposition of C1q and C5b-9, C3b and the expression of the receptors for C1q onto endothelial cells was enhanced by CSE and nicotine-containing ECVE while C4d were increased by CSE only. Since complement deposition onto endothelial cells is known to promote CVD progression, both CS and E-cigarette may increase the risk of CVD. Inhibition of endothelial cell migration is a mechanistic link between CS and CVD. CS suppressed HUVEC migration dose-dependently but ECVE did not exert this effect.[63] The abundance of oxidants and free radicals in CS can lead to oxidative damage, apoptosis and necrosis in endothelium, and this paper, in contrast, proposed that E-cigarette use may reduce the risk of CVD by reducing exposure to oxidant species in users.[63] Myocardial cells are another example of cells in which oxidative stress is induced by CS. A rat myocardial cell line exposed to CS extract suffered large-scale cell death and morphological changes; by contrast, ECV extract produced from only a minority of brands of E-cigarette caused significant cell death, especially those with tobacco flavouring, raising the possibility that several tobacco impurities may be present in these E-liquids.[64] Indeed, nicotine and humectants alone did not induce cytotoxicity, suggesting that flavourings and other components in E-cigarettes play a significant role in causing harm (see below).

Kupffer cells are liver-resident macrophages that locate to the liver sinusoids. They clear foreign substances by adhering to platelets, which then return to the systemic circulation. Some studies indicate that liver inflammation might cause alterations in the circulation, leading to the development of systemic diseases. Exposure of immortalized rat Kupffer cells to CS, ECV extract or nicotine significantly decreased cell viability and density.[65] The deposition of complement proteins C1q, C4d, C3b and C5b-9 on the Kupffer cells was augmented by ECVE, CS and nicotine. As C5b-9 is the main lytic moiety of the complement cascade, the authors hypothesized that increased C5b-9 deposition on Kupffer cells might be one reason for the reduction of Kupffer cell viability. Elevated expression of C1q receptors (C1qR) on Kupffer cells was also observed. No significant differences were observed between the effects of CS and ECV extract or various concentrations of nicotine in ECV extract. In addition, although nicotine alone was detrimental to these cells, E-cigarettes further enhanced the effects, suggesting that other components in E-cigarettes initiate inflammatory responses. ECVE also induced ROS and pro-inflammatory cytokine secretion by the Kupffer cells.

2.5 | Inflammatory effects of E-cigarettes in murine models in vivo

Increased airborne levels of nicotine and cotinine in homes of E-cigarette users are associated with passive exposure to ECVs (Figure 3 and Table 4).[17] Children may absorb higher amounts of ECV constituents than adults because of their higher respiratory rates and increased dermal absorption; in addition, they have diminished nicotine clearance.[18] Murine experiments have therefore been performed to better understand the potential effects of ECV constituents in the young. Newborn mice exposed to ECV containing nicotine for 10 days after birth had significantly lower body-weight, and there was a positive correlation between reduction of body-weight and plasma cotinine levels.[66] Nicotine-containing ECV also attenuated alveolar growth in neonatal mice, assessed by a significant reduction in expression of the cell proliferation marker Ki67.[66] Exposure of mice to CS has also been reported to lead to reduced body-weight[67] and lung development.[68]

Acute lung injury and greater IL-6 secretion were observed in adult mice after 3 days of CS exposure and, to a lesser extent, after ECV exposure.[69] On the other hand, only CS (and not ECV) induced oxidative stress and secretion of

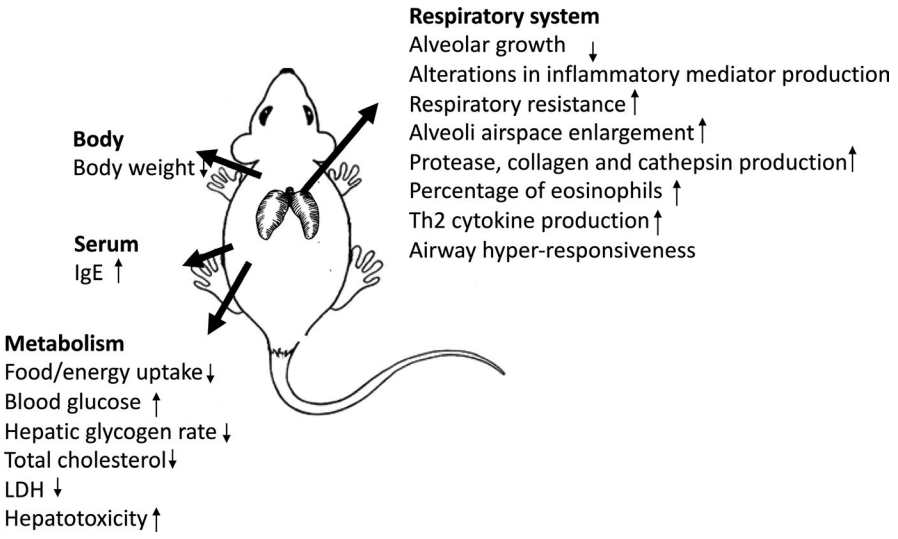

FIGURE 3 Effects of electronic cigarettes in murine models

TABLE 4 The effects of CS and E-cigarette on murine models in vivo

Effect	CS	E-cigarette
Body-weight	Lower[67]	Lower[66]
Alveolar/lung growth	Decrease[68]	Decrease[66]
IL-6 production	Increase[69,72]	Increase[69]
IL-1β production	Increase[69,76]	No[69]
TNF-α production	Increase[69,73,74]	No[69]
Oxidative stress	Increase[69]	No[69]
Other inflammatory mediators in BALF/Lung	Increase[73,74]	Increase[40,70,71]
Respiratory resistance	Increase[72]	Increase[71]
Alveolar space enlargement	Increase[73,74]	Increase[71]
Enzyme production (ie protease, collagen and cathepsin)	Increase[73,74]	Increase[71]
Airway hyper-responsiveness	Increase[77,78]	Increase[79]
Total leucocytes in BALF	Increase[77] No[78]	Increase[79]
Percentage/number of eosinophils in BALF	Increase[77,78]	Increase[79]
IgE in serum	Increase[77,78]	Increase[79]
Th2 cytokine production (ie IL-4, IL-5) in BALF	Increase[78]	Increase[79]
IL-13 production in BALF	No[77] Increase[78]	Increase[79]
IFN-γ production in BALF	Increase[80]	Decrease[79]
Food/energy uptake	Decrease[67]	Decrease[81]
Blood glucose	N/A	Increase[81]
Hepatic glycogen rate	N/A	Decrease[81]
Total cholesterol	N/A	Decrease[81]
LDL release	N/A	Decrease[81]
Hepatotoxicity	N/A	Increase[81]

BALF: bronchoalveolar lavage fluid; CS: cigarette smoke; E-cigarette: electronic cigarette; IL: interleukin; IFN-γ: interferon-γ; LDL: low-density lipoprotein.

IL-1β and TNF-α, indicating less toxicity of E-cigarettes.[69] In other studies, mice treated with tobacco-flavoured ECVs for 3 days were observed to have significantly elevated levels of IL-6, MCP-1, IL-1α and IL-13 in bronchoalveolar lavage fluid (BALF),[40] and pentraxin-3 was elevated in mice exposed to ECV over a period of 4 weeks.[70] These data demonstrate

that acute exposure to e-cigarette aerosol in mouse lung induces inflammatory responses. Chronic exposure of mice to nicotine-containing ECV over a 4-month period induced respiratory resistance and alveolar airspace enlargement reminiscent of features of COPD,[71] as also found in CS-exposed mice.[72-74] Moreover, the nicotine-containing ECV induced increased levels of IL-1β and MCP-1 in the lungs, as well as protease, collagenase and cathepsin expression, all of which are involved in the pathogenesis of COPD. These changes were not induced by ECVs lacking nicotine, thus supporting the direct harmful effects of nicotine. All these inflammatory mediators and enzymes were similarly found to be stimulated in other experiments treating mice with CS.[72-76]

In addition to inducing COPD-like symptoms, another murine study demonstrated that both CS and E-cigarette fluid can exacerbate the signs and symptoms of experimental asthma induced by sensitization of mice to ovalbumin. Airway hyper-responsiveness was significantly increased by CS in OVA-sensitized mice[77,78] and also by intra-tracheal instillation of E-liquid twice a week for 10 weeks.[79] Furthermore, the total number of leucocytes in BALF was higher in OVA-sensitized mice also exposed to CS[77] or E-liquid,[79] as was the percentage/number of eosinophils.[77-80] CS elevated OVA-specific IgE in serum[77,78] and increased OVA-induced Th2 cytokines IL-4 and IL-5 in BALF,[78] whereas the production of IL-13 varied.[77,78] Levels of OVA-specific IgE in serum, and IL-4, IL-5 and IL-13 in BALF, were all higher in OVA-sensitized mice with E-liquid than those without, whereas interferon (IFN)-γ was lower which was contradictory to one study using CS.[80] Th2 cytokines are the crucial players in the pathogenesis of atopic asthma, and IFN-γ counteracts the Th2 immune responses; thus, a reduction in IFN-γ levels caused by E-liquid exposure might exacerbate Th2-dominated inflammatory responses. In conclusion, these results indicate that E-liquid exposure aggravated allergen-induced asthmatic inflammation and use of E-cigarettes in asthmatic people might exacerbate their symptoms.

In addition to respiratory effects, mice exposed to CS[67] or injected with nicotine and nicotine-containing E-liquid for 4 weeks[81] had significantly less food and energy intake. Furthermore, nicotine or E-liquid (with or without nicotine) elevated blood glucose levels but inhibited hepatic glycogen rate. Also, nicotine or E-liquid (with or without nicotine) might help lipid profile by decreasing total cholesterol and low-density lipoprotein (LDL) but increase hepatotoxicity. Therefore, E-liquid might induce metabolic disorder by disturbing both glucose and cholesterol homeostasis, independent of nicotine. It should be borne in mind, however, that direct application of E-liquids themselves (as in the above studies) may not be equivalent to ECV in the effects exerted. The relevance of these findings to ECV inhalation may therefore be questioned.

2.6 | Effects of E-cigarettes on resistance to infection

Platelet-activating factor receptor (PAFR) is used by pneumococci to adhere to host cells, and CS increases pneumococcal adhesion by up-regulating PAFR expression in human lower epithelial cells (Table 5).[82] The increased levels of nasal PAFR were seen in adults inhaling E-cigarette vapour for 5 minutes.[83] ECVE (with or without nicotine) also increase pneumococcal adhesion to a human epithelial cell line, and this was related to ROS production. Since pneumococcal infection is the commonest cause of bacterial pneumonia, E-cigarette use has the potential to facilitate pneumococcal diseases.

Electronic cigarette vapour may impair defence against bacterial and viral infections, resulting in increasing pathogen burden and pro-inflammatory cytokine production whilst suppressing phagocytic and defensive abilities of cells. For example, treatment of normal human tracheobronchial epithelial cells with tobacco-flavoured E-liquid, with or without nicotine, increased the virus load after human rhinovirus (HRV) infection and augmented HRV-induced IL-6 production. Moreover, SPLUNC1, a host defence molecule against HRV infection, was suppressed by E-liquids with and without nicotine.[84]

Cigarette smoke caused higher pulmonary bacterial burden of methicillin-resistant *Staphylococcus aureus* in mice, which was due to augmented evasion of phagocytosis and neutrophil extracellular trap-mediated killing.[85] With regard to immunity to bacteria, exposure to ECV prior to bacterial infection led to greater numbers of methicillin-resistant *S. aureus* in epithelial cells, alveolar macrophages and human polymorphonuclear leucocytes, which are responsible for clearance of inhaled pathogens and host defence of the lung against bacteria. This reduction in antibacterial activity was seen upon treatment with nicotine or humectants, as well as complete ECV. Increased virulence of the bacteria themselves was also induced by exposing them to ECV extract.[70]

Bacterial burden was significantly increased in the lungs of both CS-[86] and ECV-exposed[87] mice after *Streptococcus*

TABLE 5 The effects of CS and E-cigarette on resistance to infection

Effect	CS	E-cigarette
PAFR level	Increase[82]	Increase[83]
Pathogen adhesion	Increase[82]	Increase[83]
Pathogen burden	Increase[85,86]	Increase[70,84,87]
IL-6 production	N/A	Increase[84]
Host defence molecule SPLUNC1	N/A	Decrease[84]
IL-17A production	N/A	Decrease[87]

CS: cigarette smoke; E-cigarette: electronic cigarette; IL: interleukin.

pneumoniae infection, and this was associated with reduced bacterial clearance by alveolar macrophages. ECV exposure also caused greater severity of influenza infection in mice, with higher viral titres and increased mortality, but reduced levels of IL-17A which is associated with protective immunity against influenza infection.[87]

2.7 | The effects of E-liquid constituents other than nicotine

The studies described above show the potentially deleterious effects of E-cigarettes (Table 6). These effects are not entirely attributable to nicotine, indicating that other components in E-liquids may also be damaging. In addition to nicotine, humectants (ie, propylene glycol and vegetable glycerol) and flavourings are the major ingredients of E-liquids. Humectants are the bases, or carriers, for nicotine and flavourings, and most E-liquids contain both propylene glycol (PG) and vegetable glycerol (VG) at a ratio of 50/50 or 70/30. PG is widely used in food and inhaled therapeutic products and can deliver a strong throat hit which may cause a slight irritation after use. VG, which has a slightly sweet taste, can soften the feeling in the throat and generate thicker and more visible vapours. The levels of oxidants and/or ROS detected in aerosols of PG and VG were significantly higher than in air alone.[40] Both PG and VG also induced death of normal HBECs, and the effects of glycerol were more potent than complete E-liquid.[33] Moreover, cells exposed to PG or VG showed higher levels of oxidative stress than when exposed to air.[33] PG/VG mixture also had negative effects on cell viability of embryonic kidney 293 cells, human adenocarcinoma alveolar basal epithelial cells and human airway smooth muscle cells, in a dose-dependent manner.[88]

TABLE 6 The effects of E-liquid constituents other than nicotine

Effect	Other constituents and flavourings
Increase oxidative stress	Humectants,[33,40] classic tobacco,[40] menthol[40]
Alteration in cytokine production	Cinnamon roll,[40] coffee,[92] strawberry,[92] sini-cide,[94] kola,[94] cinnamaldehyde[95]
Reduce cAMP response	2,5-Dimethylpyrazine[91]
Reduce exogenous ATP response	2,5-Dimethylpyrazine[91]
Increase PKA activation	2,5-Dimethylpyrazine[91]
Reduce cell viability	Humectants,[33,88] menthol,[92] strawberry,[92] coffee,[92] Peanut butter cookie,[93] banana pudding,[93] kola,[93] hot cinnamon candies[93]
Reduce metabolic activity	Menthol,[92] strawberry,[92] coffee[92]
Reduced cell number	Peanut butter cookie,[93] banana pudding,[93] kola,[93] hot cinnamon candies[93]
Reduce cell proliferation	Captain black cigar,[93] Peanut butter cookie,[93] T-bone,[93] popcorn, black licorice,[93] energon,[93] vanilla tobacco,[93] banana pudding,[93] kola,[93] hot cinnamon candies,[93] menthol tobacco,[93] solid menthol[93]
Increase LDH release	Kola,[93] hot cinnamon candies,[93] menthol tobacco[93]
Decrease phagocytosis of alveolar macrophages	sini-cide,[94] kola[94]
Decrease phagocytosis of airway neutrophils	Hot cinnamon candies,[94] banana pudding,[94] menthol tobacco,[94] banana, sini-cide[94]
Alteration in NET formation	sini-cide,[94] kola[94]

cAMP: cyclic adenosine monophosphate; E-liquid: E-cigarette liquid; NET: neutrophil extracellular traps.

More than seven thousand flavourings for E-cigarettes are now available, with over 70% of E-liquids containing greater than 1% flavoured chemical levels by weight.[89] Although most of the flavourings are defined as safe to be used in food products, the potential toxicity associated with degradation products generated by vaping at high temperatures is unknown. Significantly elevated ROS production was discovered in classic tobacco-flavoured (16 mg of nicotine) and menthol-flavoured (0 mg of nicotine) ECVs.[40] However, classic tobacco-flavoured ECVs gave relatively reduced ROS production when nicotine was also present. Similarly, E-liquids with either high or low concentration of nicotine had significantly less ROS than those without nicotine, suggesting nicotine is not a crucial inducer of ROS production. Moreover, E-liquids with non-tobacco flavouring had higher ROS than tobacco flavoured E-liquid, indicating that the occurrence of ROS in E-liquids is associated with the type of flavourings. The levels of ROS present in fruit-flavoured ECVs can up to threefold higher than in tobacco-flavoured ECV.[90]

Flavouring also has an impact on inflammatory responses: For example, nicotine-free cinnamon roll-flavoured ECV induced high IL-8 secretion from human lung fibroblasts.[40] 2,5-Dimethylpyrazine, an odorant compound in dark chocolate, reduced the capacity of airway epithelial cells to respond to cyclic adenosine monophosphate and exogenous ATP which are crucial factors for normal airway epithelial cell functions, like mucociliary clearance.[91] 2,5-Dimethylpyrazine also increased ion conductance by evoking protein kinase A-dependent (PKA) activation of the cystic fibrosis transmembrane conductance regulator ion channel. Menthol, coffee and strawberry-flavoured ECVs reduced both cell viability and metabolic activity, while coffee and strawberry-flavoured ECVS also induced cytokine production in human bronchial cells.[92] CALU3 airway epithelial cell line proliferation was inhibited by all 13 flavoured E-liquids

tested in a dose-dependent manner.[93] Peanut butter cookies, banana pudding, kola, hot cinnamon candies and menthol tobacco caused significantly reduced cell numbers and viability compared with the PG/VG mixture. Moreover, kola, hot cinnamon candies and menthol tobacco increased production of the cytotoxic marker LDH. Aerosolized E-liquids had similar toxicity profiles to neat E-liquids. Sini-cide and kola-flavoured E-liquid altered phagocytic ability and cytokine production in alveolar macrophages.[94] Moreover, hot cinnamon candies, banana pudding, menthol tobacco and sini-cide affected phagocytosis of human airway neutrophils in comparison with PG/VG mixture. Kola and sini-cide also altered formation of neutrophil extracellular traps (NETs), which contain chromatin filaments and granule protein to expel extracellular pathogens, and killing reduced the efficiency of NK cells. These three flavourings all contained cinnamaldehyde, which, on its own, showed similar effects, suggesting the potentials of cinnamaldehyde for impairing respiratory immune cell function. Another study also pointed out the deleterious effects of cinnamaldehyde: both flavouring chemicals and flavoured E-liquid without nicotine-reduced cell viability and IL-8 secretion of monocytic cell lines dose-dependently, with cinnamaldehyde showing the highest cytotoxicity.[95] Flavouring chemicals and seven types of aerosolized E-liquids including American tobacco, café latte and cinnamon roll induced cell-free ROS production.[95] Interestingly, mixing a variety of flavours induced higher cytotoxicity and cell-free ROS levels compared to individual flavours. This might give a warning of greater toxic effects to those users who mix E-liquids or those who are exposed to multiple flavourings in public areas.

A comprehensive study examined the effects of 35 flavours of E-liquid on human embryonic stem cells (hESC), mouse neural stem cells (mNSC) and human pulmonary fibroblasts (hPF).[96] Cinnamon Ceylon flavour was the most cytotoxic to all three cell types, whilst butterscotch was defined as having relatively low cytotoxicity. However, the possibility of harm being caused by the latter cannot be excluded, since some workers at microwave popcorn packaging plants developed the irreversible obstructive lung disease, bronchiolitis obliterans, due to exposure to the butter flavouring component diacetyl.[97] Three E-liquids all named butterscotch flavour induced different levels of cytotoxicity, although they were from the same company. hESC and mNSC were more sensitive to E-liquids than hPF (except for caramel and Menthol Arctic, which had stronger effects on hPF), suggesting that cells from embryos and newborns are generally more sensitive to E-cigarette constituents. There was no correlation between cytotoxicity and nicotine levels, as the cells did not survive better in E-liquids without nicotine.

In addition to humectants and flavourings, other chemicals and metals (eg, copper, nickel and titanium) are detected in E-liquids. Copper metal nanoparticles could stimulate the production of mitochondrial ROS and reduction of mitochondrial membrane potential in human lung fibroblasts.[40] A quarter of E-liquids contain aldehyde (eg, benzaldehyde and vanillin)[89] and significant amounts of formaldehyde, acetaldehyde and propionaldehyde were found in E-liquid heated at 150°C.[98] Aldehydes are recognized as primary irritants of the mucosa of the respiratory tract and acetaldehyde exposure impairs mucus clearance, leading to decreased host defence. Benzaldehyde, which can cause irritation of the respiratory airways and eyes, is a major ingredient in fruit-flavourings. Cherry-flavoured E-liquid was found to contain the greatest concentration of benzaldehyde, and the benzaldehyde doses inhaled with 30 puffs from E-cigarettes were often higher than doses inhaled from conventional cigarettes.[99] Tobacco-specific nitrosamines (TSNAs) are known carcinogenic compounds in conventional cigarettes, especially NNK and NNN, which exert the strongest carcinogenicity. TSNAs were also detected in both liquid[100] and vapour[10] of E-cigarettes. However, conventional cigarettes contain up to 1800 times higher amounts of NNK and NNN than E-cigarettes.[101]

3 | DISCUSSION

Electronic cigarettes are battery-operated devices that generate vapours by heating liquid mainly containing humectants, nicotine and flavourings. E-cigarettes have seen a rapid rise in use since the introduction to the market because of the perception that they are safer than conventional cigarettes, and their appealing design; however, there are many unanswered questions regarding their health impacts on humans. There is accumulating evidence demonstrating the effects of E-cigarettes on both human and murine models, and comparison between E-cigarette and conventional cigarettes has also been made. With respect to the latter, numerous studies have indicated that ECV can induce similar effects to CS, but to a lesser extent. This may mean that smoking E-cigarettes is unlikely to be as deleterious to health as smoking conventional cigarettes; however, the relatively recent widespread introduction of E-cigarettes means that it is too early to know the potential consequences, for an individual, of using E-cigarettes for several decades. A very important aspect of this is the carcinogenic potential of E-cigarettes compared to conventional cigarettes. Once again, however, it is too early to draw conclusions about the actual carcinogenicity of ECVs.

Gingival epithelial cells, the most abundant structural cells in gingiva, are in direct contact with vapours from smoking devices. As described in previous section, both CS and ECV induced death of gingival epithelial cells, associated with the elevation of caspase-3 activity, which is a frequently activated protease in cell apoptosis. Moreover, ECV altered the morphology of the cells, resulting in large faint nuclei, enlarged cytoplasm and compromised integrity of

the plasma membrane. Gingival fibroblasts are important in wound healing through their adhesion, proliferation and migration, which were affected negatively by both CS and ECV. As CS is a well-established risk factor for periodontitis, these findings indicate that ECV might also provide an unhealthy oral environment and promote periodontal disease. Indeed, two clinical cases were reported of patients with more than a 10-year history of E-cigarette use, but no other identifiable risk factors, developed basaloid squamous cell oral carcinoma, suggesting a potential role for ECV in oral cancer development.[102]

A clinical case report described a previously healthy individual who started coughing, with whitish secretions, and who developed progressive dyspnoea after E-cigarette use for 48 hours; wheezing was revealed by auscultation after 4 weeks. However, these symptoms improved gradually and were abolished without any treatment a week after cessation of E-cigarette use.[103] Another case report described a presentation of hypersensitivity pneumonitis associated with E-cigarette use.[104] Also, a 46-year-old healthy man developed respiratory distress, night sweats, fever and weight loss after using E-cigarette for a month. He was found to have BALF that contained abundant lipid-laden macrophages, eosinophils and neutrophils. The symptoms were fully abolished after E-cigarette abstinence.[105] These clinical cases illustrate that E-cigarettes can have a negative impact on respiratory health. Respiratory epithelium is a ciliated epithelium forming the integral barrier against inhaled agents to prevent infection and injury by trapping and expelling particles (mucociliary clearance), phagocytosing and killing pathogens and recruiting immune cells. It is known that CS alters respiratory epithelium by compromising its integrity and impairing mucociliary clearance. According to the findings described in a previous section about respiratory system, ECV also induced morphological changes, cell death, LDH release and inflammatory mediator production by bronchial epithelial cells. Furthermore, ECV might compromise mucociliary clearance by down-regulating the expression of genes related to assembly and movement of cilia. Although some studies observed a lower magnitude of effects than those caused by CS, ECV is also able to damage respiratory epithelium and evoke an inflammatory environment. Using human models, both direct exposure and passive ECV exposure were found to facilitate adverse respiratory effects including alterations in transcriptome/protein expression related to various biological functions, increased bronchitic symptoms, airway flow resistance and oxidative stress measured by FeNO.

Oxidative stress, which reflects an imbalance between oxidants and antioxidants, is critical in injurious and inflammatory responses in respiratory diseases.[106] Oxidative stress causes damage to airway epithelium by increasing epithelial cell lysis and detachment after CS exposure, and triggers the release of inflammatory mediators such as IL-1 and IL-8 which are involved in activation of the NF-κB pathway. Tobacco smokers have higher levels of oxidative stress in the lungs, which arises not only from oxidants in cigarettes, but also ROS from macrophages and neutrophils via the NADPH system. Excessive ROS damage cells and tissue, leading to induction of cell death.[107] These mechanisms might also explain oxidative stress-induced deleterious effects of E-cigarettes since ECV causes high levels of oxidative stress in a variety of cell types, as discussed in previous sections.

Alveolar macrophages are crucial in innate immune responses in lungs. As mentioned in previous sections, both CS and ECV induced the production of pro-inflammatory mediators and proteolytic enzymes which damage connective tissues and parenchymal cells of lungs, thereby providing cellular mechanisms that connect smoking/vaping and inflammation and tissue damage. Moreover, increased ROS production can help the killing of intracellular pathogens; however, phagocytosis of alveolar macrophages was compromised by both CS and ECV, suggesting their roles in impairing defence ability. Excessive inflammatory mediators and functional impairment of alveolar macrophages could contribute to lung pathogenesis including chronic bronchitis and emphysema. In previous sections, both CS and ECV reduced the production of inflammatory mediators associated with an anti-viral response by bronchial epithelial cells, showing that both CS and ECV might increase susceptibility to infection, which is the main factor in the acute exacerbation of COPD. It was reported in animal models that chronic exposure to CS suppressed bacterial clearance from the lungs and induced COPD-like damage in the lungs. ECV also impaired resistance to infection in mice, leading to higher burden and adhesion of pathogens, which might be due to inhibition of defence mechanism (ie lower levels of SPLUNC1, IL-17 and clearance activity by leucocytes).

Cigarette smoke has been documented to have a negative impact on many aspects of human health and to be a risk factor for various diseases. In particular, tobacco smoking is a risk factor for a range of cancers, respiratory diseases and infections, cardiovascular diseases and deleterious effects on the immune system.[108,109] ECV also exerts systemic effects on various cells and tissues. Neutrophils are recruited to the lung and stimulate enzymes that break down collagen, elastin and extracellular matrix and mucus production after CS exposure, leading to the development of emphysema and chronic bronchitis, major characteristics of COPD. In addition, smokers who developed COPD have higher numbers of circulating neutrophils.[110] We have described in previous sections that sputum of E-cigarette users has a raised neutrophil count, neutrophilic enzyme production and NET formation, which promote antimicrobial ability. Moreover, ECV could activate neutrophils and inflammatory mediator production in vitro. ECV might be harmful to the cardiovascular system as it was found to

reduce endothelial functions, decrease cell viability and induce oxidative stress and complement deposition: All these factors could promote the development of cardiovascular disease. Habitual E-cigarette users showed sympathetic predominance and higher oxidative stress levels. Viability and density of Kupffer cells were decreased by ECV while complement deposition, oxidative stress and inflammatory mediators were induced, suggesting that ECV might trigger inflammation in the liver. In murine models, ECV was able to affect not only respiratory system, but also body growth, energy intake and metabolism.

A clinical case report described an E-cigarette user who developed respiratory symptoms that coincided with her onset of E-cigarette use and was given a diagnosis of lipid pneumonia.[111] Lipid pneumonia results from accumulation of either exogenous or endogenous lipids in the alveoli. Glycerine is obtained from plants by heating palm or coconut oils or soap via a fatty acid splitting operation; therefore, glycerine-based oil in E-cigarettes was suspected to be the exogenous source of lipids that induced lipid pneumonia in this individual. Flavourings have increased the appeal of E-cigarettes to adolescents and previous intermittent smokers; the FDA planned to limit sale of flavoured E-cigarette to crackdown the "epidemic" use of E-cigarettes amongst the young.[16] Indeed, as discussed in previous sections, many flavoured E-cigarettes had adverse effects in vitro, and there are many more E-cigarette flavourings on the market waiting to be investigated. Although humectants, nicotine and flavourings are labelled as the ingredients in most commercial E-liquids, metals and other chemicals such as carbonyls and tobacco-specific nitrosamines, which are toxic and carcinogenic, have been detected in E-liquids.

There are limitations studying the effects of E-cigarettes, such as types of E-cigarette devices and E-liquid used and patterns of E-cigarette exposure (ie duration and frequency). Quantifying E-cigarette exposure is more challenging than conventional cigarette exposure, which can be calculated by the number of cigarettes smoked per day. In those human studies, it is also difficult to certain that the participants did not use conventional cigarette surreptitiously or have been exposed to CS-containing environment.

4 | CONCLUSION

Electronic cigarette vapours induce many alterations in a variety of cell types, including suppression of cell viability, morphological changes, production of pro-inflammatory cytokines and inhibition of defence against bacteria and viruses. In addition to in vitro models, murine and human in vivo studies also demonstrate potentially deleterious effects of E-cigarettes, indicating they may not be as safe as initially assumed and have effects like those of CS, although possibly less severe; a caveat to this is that it is still too early to know what effects E-cigarettes may have in an individual over several decades of use. The effects of E-cigarettes are attributable not only to nicotine but also to humectants, different flavourings and other constituents. In conclusion, we cannot neglect the potential negative health impact of E-cigarettes and the need for public awareness of this.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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