



Review

The impact of e-cigarette exposure on different organ systems: A review of recent evidence and future perspectives

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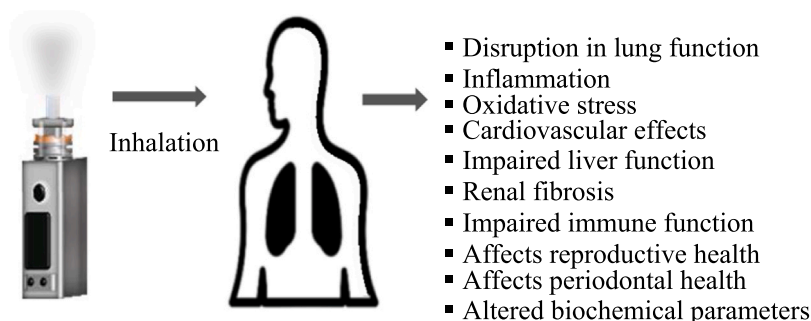


HIGHLIGHTS

- Long-term exposure to e-cigs may cause adverse effects on various organ systems.
- Data on the prolonged use of e-cigs use among people with pre-existing chronic diseases are missing.
- Well-supportive data regarding the usefulness of e-cigs as a smoking cessation tool are missing.
- Clinical and mechanistic studies are needed to improve the understanding of its potential health effects.

GRAPHICAL ABSTRACT

Potential adverse effects of e-cigs on various organ systems.



ARTICLE INFO

Editor: Yuming Guo

Keywords:

Electronic cigarettes
Exposure
Health effects
Organ system

ABSTRACT

The use of electronic cigarettes (e-cigs) is rapidly increasing worldwide and is promoted as a smoking cessation tool. The impact of traditional cigs on human health has been well-defined in both animal and human studies. In contrast, little is known about the adverse effects of e-cigs exposure on human health. This review summarizes the impact of e-cigs exposure on different organ systems based on the rapidly expanding recent evidence from experimental and human studies. A number of growing studies have shown the adverse effects of e-cigs exposure on various organ systems. The summarized data in this review indicate that while e-cigs use causes less adverse effects on different organs compared to traditional cigs, its long-term exposure may lead to serious health effects. Data on short-term organ effects are limited and there is no sufficient evidence on long-term organ effects. Moreover, the adverse effects of secondhand and third hand e-cigs vapour exposure have not been thoroughly investigated in previous studies. Although some studies demonstrated e-cigs used as a smoking cessation tool, there is a lack of strong evidence to support it. While some researchers suggested e-cigs as a safer alternative to tobacco smoking, their long-term exposure health effects remain largely unknown. Therefore, more

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<https://doi.org/10.1016/j.jhazmat.2023.131828>

Received 9 February 2023; Received in revised form 22 May 2023; Accepted 8 June 2023

Available online 12 June 2023

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epidemiological and prospective studies including mechanistic studies are needed to address the potential adverse health effects of e-cigs to draw a firm conclusion about their safe use. A wide variation in e-cigs products and the lack of standardized testing methods are the major barriers to evaluating the existing data. Specific regulatory guidelines for both e-cigs components and the manufacturing process may be effective to protect consumer health.

1. Introduction

E-cigarettes (e-cigs) are products that deliver a nicotine-containing aerosol (also known as vapour) to users by heating a solution generally made up of nicotine, glycerol, or propylene glycol and flavouring chemicals. Since its invention in the early 2000 s by Hon Lik [1], the use of e-cigs has increased rapidly worldwide, with a high prevalence particularly in the US and UK [1,2]. Indeed, e-cigs have received great attention due to their supposed safety and potential alternative to conventional cigs smoking [3]. While studies indicate that e-cigs deliver lower concentrations of toxic compounds than combustible cigs [4], the associated health risks of e-cigs exposure are still a matter of ongoing studies. The lung is the primary target organ of e-cigs exposure and can cause acute and long-term respiratory outcomes [5,6]. Other effects of e-cigs include nausea, vomiting, mouth irritation, headache, impacts on memory and attention, hypertension, inflammation, and oxidative stress [7–10]. The effects of e-cigs exposure, especially chronic exposure on human health are not well known yet.

Recent reports suggest that e-cigs can be useful to reduce or quit smoking, more in particular, traditional heated tobacco. For instance, a recent Cochrane review indicated that e-cigs are more useful to quit smoking than traditional nicotine replacement therapies (NRT) [11]. However, a recent European Respiratory Society task force indicated that the long-term effects of e-cigs are largely unknown, therefore, it is still unclear whether using e-cigs is a safer alternative to conventional cigs and according to current knowledge, its adverse health effects cannot be ruled out [12]. This review aims to summarize the impact of e-cigs on the lungs and its subsequent effects on other organs (multi-organ systems) based on the rapidly expanding recent evidence from experimental and human studies, to highlight the research gaps and to provide further directions for future studies. To present a clear overview of the health impact of e-cigs, we categorized their effects according to the organ system.

2. Methods

To identify relevant and recent studies for this review, we relied upon scientific databases such as Science Direct, PubMed/Medline, and Google Scholar applying the following keywords: electronic cigarettes or 'electronic nicotine delivery systems or e-cigs AND [health OR adverse effects OR toxicity, respiratory system OR cardiovascular system or hepatic system OR oral health OR renal system, gastrointestinal system OR auditory system OR reproductive system, OR endocrine system OR musculoskeletal system, OR nervous system OR immune system OR smoking cessation and safety]. All article titles and abstracts were screened to select relevant studies with full-text availability. For relevant literature review and primary studies, we also browsed the reference list of eligible literature.

3. E-cigs as a smoking cessation tool

E-cigs manufacturing companies often claim that e-cigs use does not cause adverse health effects, moreover, it helps smokers to reduce or quit cig use. Several studies reported that e-cigs can be useful to reduce or quit cig use. For example, a cross-sectional study indicated that long-term NRT-only and e-cigs-only use, but not dual use of NRTs or e-cigs with combustible cigs, was associated with reduced levels of carcinogens and toxins in urines relative to smoking only combustible cigs [13].

According to a randomized control trial switching from traditional cigs to e-cigs was associated with a significant improvement in endothelial function and vascular stiffness [14]. The Committee on Toxicity (COT) also indicated that switching from combustible smoke to e-cig may aid in smoking cessation [15]. Nicotine vaping in England: new evidence suggested that alternative nicotine delivery devices such as vaping products can play a vital role in decreasing the great health burden caused by traditional cig smoking [16]. Further, a recent Cochrane review indicated that e-cigs may help people to quit smoking significantly more than traditional NRT [11]. A cross-sectional survey in Great Britain in 2022 reported that regular e-cigs use is more frequent than smoking in children and youth, though a major number of them were also used to traditional cigs smoking [17].

Some small studies also reported a short-term reduction in cig smoking while using e-cigs [18–21]. Smokers also reported fewer withdrawal symptoms during e-cigs use while quitting [18,22]. A recent randomized trial that included participants who wanted to quit smoking willingly [23], showed that the abstinence rate was about two times higher in the e-cigs group compared to the nicotine-replacement group (18.0% vs. 9.9%) after 1 year. However, the incidence of mouth and throat irritation was higher in the e-cigs group compared to the nicotine-replacement group (65.3% vs. 51.2%, respectively) [23]. As studies conducted to assess the efficacy of e-cigs as a cessation tool showed mixed results, with low sustained cessation rates (verified or self-reported) [19,24,25], further studies, especially with longitudinal design are required to determine the efficacy of e-cigs as a smoking cessation therapy. Based on the above findings it can be concluded that using e-cig may be useful for smoking cessation. However, even e-cigs are comparatively safer than conventional smoking but not without risks. The supporting data are still insufficient. According to the World Health Organization (WHO), e-cigs cannot be an effective method to quit smoking unless there is strong evidence [26,27]. Indeed, there are inconsistent reports regarding the use of e-cigs as a smoking cessation tool [23,28–30]. The severe impact of e-cigs on the respiratory system is still being investigated by researchers and regulatory bodies including the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) [31].

4. Components of e-cigs

A variety of e-cigs models have risen over the past two decades. Although there are some differences in design and features between the e-cigs models, the basic principle is the same. A typical e-cigs consist mainly of three parts such as a rechargeable lithium battery, a reservoir for e-liquid, and an atomizer (Fig. 1). When the heating coil within the atomizer is activated, nicotine aerosol is generated (also called e-vapour). In recent years, the use of lithium-ion batteries has also been found to cause an increased rate of e-cigs explosion-related injuries [32]. E-cigs are either disposable or can be used multiple times in which case, the container or reservoir is required to be refilled with e-liquid or replaced with a prefilled cartridge. The e-liquid consists of nicotine, flavourings (tobacco, mint, fruit or dessert) and one to two solvents like vegetable glycerin (VG) and propylene glycol (PG) [33].

In e-cigs, some potential toxic compounds may be present, for example, heavy metals (lead, mercury, copper, silver, and cadmium), carbonyl components (acetaldehyde, formaldehyde, and acrolein) and volatile compounds (toluene, benzene) [34,35]. Other components, like vitamin E acetate, is sometimes used as an additive in e-cigs, most

commonly in those containing tetrahydrocannabinol (THC). Studies indicate a lower level of particulate matter (PM) in e-cigs than in conventional cigs but the levels are still higher than that described in WHO guidelines [36]. The inhalation effects of e-cigs depend on several factors like airflow, room size, climate condition, number of users in the vicinity, and characteristics of the user (eg. sex, age, and health status). Pulmonary absorption of e-cigs aerosol particles depends on their size and products, although, details about the aerosol particles and pulmonary absorptions are not well known [37].

The general ingredients of an e-cigarette mixture include VG, PG, flavouring agents, and various proportions of nicotine. Different research has been carried out to determine the potential toxicity associated with the constituents of the e-cig mixture. PG and VG are the primary vaporising solvents present in the e-cig mixture [38]. They are considered humectants or delivery solvents, and they help maintain the moisture contents in the filler [39]. The colourless and odourless PG is used in the food industry as a solvent for various flavouring and colouring agents and in the medicinal industry as a solvent for drugs and cough syrups. The aerosolized exposure to PG can cause upper respiratory irritation [40]. Generation and accumulation of PG and VG-derived fats have also been suggested to cause lipoid pneumonia, a disease state where alveoli are filled with lipid-laden macrophages with or without inflammation [41]. Repeated high-dose intravenous administration can result in side effects such as hemolysis, hyperosmolarity, lactic acidosis, and cardiac arrhythmia [42]. PG can absorb orally, through skin or mucus membranes and is primarily eliminated through the kidney and liver. Therefore, patients with impaired kidney or liver function are at high risk of developing PG-related toxicity [43]. Due to the wide use of PG in the food industry, various in-vivo studies have been performed to identify and characterize the toxicological effects. Most of the studies demonstrated the non-toxic nature of PG. However, very few were shown potential side effects, including alteration of goblet cells and ciliated epithelial cells of rabbit upon the aerosol of exposure PG (10% aerosol of PG) [44], impaired kidney function in dogs upon chronic exposure (5000 mg/kg/day for 2year) [45].

PG and VG are generally used as skin moisturisers and are known as upper airway irritants [46]. Contact with glycol mist may also cause drying out of eyes and mucus membranes [46,47]. In contrast, glycerine has some therapeutic applications. However, there is not enough data to estimate the long-term safety of glycerin used. The other important component of e-cigs is nicotine which is easily absorbed via the airway, skin, mucous membrane and GI tract.

The nicotine concentration in the e-cig can vary among products; also, the presence of nicotine in the e-cig mixture is optional [48]. Usually, the concentration is varied from 0 to 87.2 mg/ml [49]. The strength of the nicotine can be influenced by the specification of the e-cig, including the power and the user behaviour (puff duration and puff frequency) [48]. Based on product features and puff conditions 15 puffs from a single e-cigs can provide far less or far more nicotine than one conventional cig [48]. Many research groups have studied the pharmacology and toxicity associated with nicotine exposure. Upon the inhalation of nicotine-containing, e-cig-generated puff, the nicotine will quickly absorb into the pulmonary venous circulation and be translocated to the brain through the arterial circulation via crossing the blood-brain barrier [50]. Once nicotine reaches the brain, it will bind to the ligand-gated ion channel, nicotine cholinergic receptor (nAChRs). The stimulation of nAChRs by nicotine leads to the release of various neurotransmitters in the brain, such as dopamine, norepinephrine, acetylcholine, serotonin, endorphins, glutamate etc [51]. Even though the current studies do not wholly support nicotine as a carcinogen, there is some evidence to support the cancer risk associated with nicotine exposure. Acute exposure to nicotine through inhalation may cause nausea, dizziness or vomiting. Nicotine can influence apoptosis inhibition, epidermal growth factor release, stimulation of fibroblast production, and metastases [52]. Exposure to nicotine increases blood pressure and heart rate, endothelial remodelling, thrombogenesis, angiogenesis, myocardial remodelling, and arrhythmogenesis [53,54]. Data on serious poisoning of nicotine through e-cigs are relatively scarce. However, increased nicotine concentrations in e-cigs may have toxic health effects [55]. Cinnamaldehyde flavouring chemicals can impair respiratory

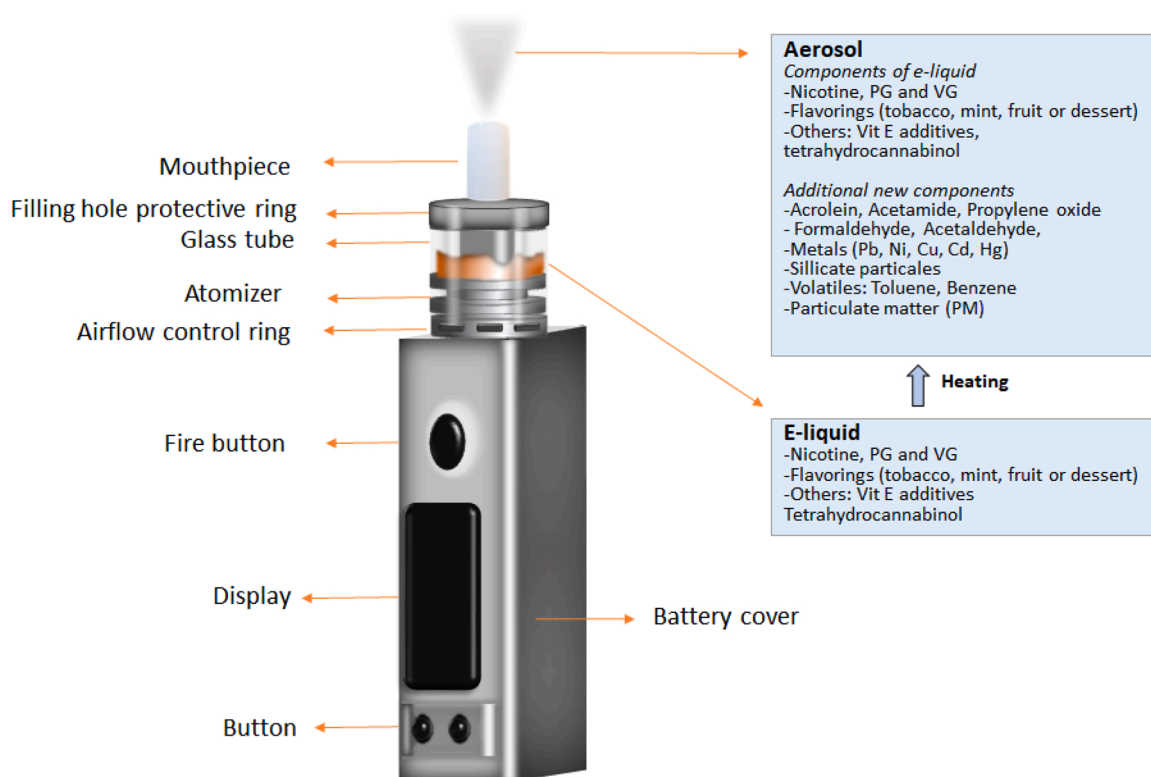


Fig. 1. A typical structure of an e-cig and its components.

immune cell function by impairing the phagocytic activity of macrophages and neutrophils and suppressing the killing of cells by NK cells [56]. Cinnamaldehyde was also found to cause impair the epithelial barrier function in 16-HBE cells [8].

5. Effects of e-cigs on different organ systems

The effects of e-cigs exposure on various organ systems have been described below and major studies are summarized in Table 1 and depicted in Fig. 2.

5.1. Respiratory system

The impact of e-cigs on the respiratory system has been well-defined in both animal and human studies. E-cigs exposure can change the lung's physiology and impair its functions. Deep into the lungs, where the gas exchange units (alveoli) are, the first barrier vapors will interact with is called lung surfactant [57,58]. Lung surfactant function is highly sensitive to compositional changes and inhibition by undesirable increased levels of proteins, lipids, and inhaled toxicants [59–62]. E-cigs vapour has been suggested to cause alteration in surfactant composition, and function leading to abnormalities in pulmonary gas exchange [63–66]. This was observed in an animal model, where e-liquid disrupted the surfactant layer and gas exchange [65]. Pulmonary gas exchange is an important mechanism of lung function that relies on tissue elastic properties, surfactant, and alveolar stability [67]. Its disruption impact lung function either acutely or chronically. In a randomized trial, gas exchange disturbance with reduced lung function was observed in humans after inhaling fourth-generation e-cigs vapour [68]. These findings highlight the potential impact of e-cigs relating to hypercarbia and hypoxia. Spirometry is a well-known method of determining lung volume and function. Studies demonstrated that e-cigs can impair lung function. A reduced lung functional residual capacity was found in e-cigs vapour exposed mice vs the non-exposed group [69]. Similarly in human studies, increased peripheral airway resistance was noticed after exposure to e-cigs vapour for 5–10 min [70]. Further, a recent study in occasional and heavier smokers demonstrated that a 60 W e-cigs aerosol exposure for 15 min caused a disturbance in gas exchange and expiratory gas flows, with elevated lung-specific protein (CC16) in the blood [68]. These results suggest that e-cigs exposure may cause acute effects and visible injury in the airways. Vitamin E-acetate, sometimes used in e-cig, has been reported as a potential toxin by CDC, due to its present in the lungs for prolong period and therefore leads to complications in the lungs and may be associated with EVALI (electronic cigarette or vaping product use-associated lung injury) [71].

The epithelial cells surface of the respiratory tract work as an active interface between external stimuli and the host immune response. It connects the respiratory tract from the nose to the alveoli, with different types of epithelial cells. Studies suggested that acute exposure to e-cigs may lead to impaired mucociliary in vivo models [72]. In-vitro studies also reported mucociliary dysfunction in the nasal mucosa and bronchial epithelial cells [73,74]. In a recent study, primary human bronchial epithelial cells cultured to an air-liquid interface and exposed to e-cigs vapour for 15 min/day for 3–5 days showed a reduced airway barrier function [75]. Studies also suggested that e-cigs exposure may reduce endothelial cell's electrical resistance obtained from rats, mice, and humans and this alteration are close to those found in conventional cigs exposure [76]. Lactate dehydrogenase (LDH) level is often determined in cell cytotoxicity assays as an indication of cell death. In a study by Cervellati et al. alveolar epithelial cells showed increased levels of LDH release when exposed to e-cigs extract and compared to those of non-exposed control [77]. In another study, a dose-dependent release of LDH was observed when A549 cells were exposed to e-cigs extract or e-cigs aerosol, and high levels of LDH were indicative of necrotic cell death [78].

There is also evidence that e-cigs exposure may cause direct lung

parenchymal damage. Epithelial membrane protein (EMP), is generally found in human serum and represents cell turnover and homeostasis at its baseline levels. A significantly high level of plasma EMP was found in e-cigs users when compared to healthy controls, suggesting a potential link between e-cigs exposure and lung cell damage through dysregulation in lung homeostasis [79]. Increased secretion of fibroblast growth factor is an indicator of diffuse alveolar damage (DAD) [77], which is frequently present in individuals with acute respiratory distress syndrome (ARDS) and associated with alveolar haemorrhage and inflammation. Indeed, DAD has been reported in a case study with a chronically vapoured female adult [80]. An enlargement of the alveolar lining and thickening of the alveolar septa were noticed when her lung biopsy was done [80].

The respiratory system has a rich microbiota, which is important to maintain pulmonary homeostasis. However, the balance between airway mucosa and microbial environment may be disrupted, and several factors are associated with it including air pollution and cigs smoke. This can cause inflammation and alter epithelial functions, which eventually leads to the spreading of a possible pathogen, resulting in infection [81]. Emerging evidence suggests that e-cigs exposure can increase the susceptibility to respiratory infection by enhancing the microbial ability for cell invasion. A study showed that e-cigs exposure increased the expression of platelet-activating factor receptors (PAFR) [82]. This study also indicated that exposure to e-cigs caused increased adhesion of bacterial communities to upper and lower airway cells. Exposure to e-cigs extract has been shown to increase viral infection in primary human airway cells in vitro [83]. In another study, e-cigs-exposed mice infected with the influenza virus showed increased tissue damage and lung inflammation and decreased cellular immune response [65]. These studies suggest that e-liquids can increase microbial adherence and decrease pulmonary clearance, resulting in a disruption in pulmonary homeostasis, and possible vapours that influence respiratory infections.

There is also clear evidence that e-cigs exposure can suppress the function of immune cells. Alveolar macrophages (AMs) is well defined in providing the initial defense in the lower airways against invading foreign particles through phagocytosis. Analysis of AM from healthy individuals reported impaired phagocytosis following e-cigs extract exposure in vitro [84]. In macrophage cell line studies, reduced phagocytosis was observed in e-liquids exposure [85]. Furthermore, transcriptome analysis of AMs from e-cigs users showed an alteration in gene expression, including genes associated with immunity and inflammation [79]. Similarly, e-cigs exposure may also affect neutrophil function. Neutrophils play a key role in removing and controlling lung infections through reactive oxygen species (ROS) and proteases released at the infection site. Analysis of e-cigs users' sputum showed a significant increase in neutrophil extracellular trap-related and neutrophil granulocyte-related proteins to those of controls [86]. In addition, e-cigs vapour exposure has also been reported to be associated with reduced chemotaxis and antimicrobial function in vitro [78,87].

Inflammation and oxidative stress in the airways are the major reasons for respiratory chronic illnesses such as chronic obstructive pulmonary disease (COPD), asthma and infections such as pneumonia. Inflammatory cytokines are associated with these chronic processes. In a murine study, chronic exposure of mice to e-cigs vapour for 1 h/day over 4 months course showed some features like COPD in humans, including hyperactivity in the airways, damage of lung tissue and elevated expression of pro-inflammatory cytokines (IL-8) in lungs [88]. In this study the effects of inhaled nicotine in e-cigs were found to be associated with the pathogenesis and progression of COPD: mucous cell hypertrophy, airspace enlargement, releasing of inflammatory molecules and altered epithelial ciliary function [88]. In a large representative study in South Korea, dual uses of conventional cigs and e-cig were found to increase the risk of COPD and the risk was higher in the older male population [89]. Another study in the USA showed that regular or occasional uses of e-cigs were associated with an increased risk of having

Table 1

Potential effects of e-cigs exposure on different organ systems (major studies in humans and animals).

Organ system	Species (human/animal)	Study design/methods	Exposure	Effects	Reference
Respiratory system	Human	A randomized, single-blind study (25 young tobacco smokers and 20 heavy smokers). Participants were not used to smoking traditional cigs (>20) per week and did not use nicotine replacement therapy	Participants were randomly assigned to sham-vaping, vaping with and without nicotine groups. E-liquid contained nicotine at 3 mg/ml. 60 W e-cigs aerosol exposed for 15 min	- Increased serum levels of club-cell protein-16 - Decreased transcutaneous oxygen tension - Decreased arterial oxygen tension	[68]
	Human	30 healthy smokers (experimental group, n = 30 and control group, n = 10)	Used an e-cig for 5 min	Increased flow airway resistance	[70]
	Human	Primary human bronchial epithelial cells, in vitro study design	Exposed to E-cigs vapour for 15 min daily for 3–5 days	- Secreted higher IL-8 in response to infection - Reduced airway barrier function - Increased LDH release. Cytotoxicity was higher in the traditional cigs group	[75]
	Human	Lung (A549) cells, in vitro study design	Exposed to e-cigs vapour and traditional cigs for 50 min using an in vitro system	Increased LDH release. Cytotoxicity was higher in the traditional cigs group	[77]
	Human	10 never smokers. Lung function, plasma endothelial microparticles (EMP) and other parameters were measured at baseline and one week later to measure biological responses in healthy and naive subjects	Subjects inhaled 10 puffs of E-cigs, and then another 10 puffs after 30 min; n = 7 were in the e-cigs with nicotine and n = 3 were in the nicotine-free e-cigs group	- Altered transcriptomes of small airway epithelium (SAE) and alveolar macrophages (AM) - Increased plasma EMP levels observed in nicotine-containing e-cigs group	[79]
	Human	A 47-year-old woman, case study	Used to smoking e-cigs for 3 years before diagnosis	Observed diffuse alveolar damage (DAD)	[80]
	Human	Human tracheobronchial epithelial (hTBE) cells received from young healthy non-smokers	Cells were treated with medium, e-liquid at different concentrations without nicotine or with nicotine (18 mg/ml) for 24 and 48 h	E-liquid (without nicotine) promoted IL-6 production and virus infection (human rhinovirus: HRV)	[83]
	Human	Human alveolar macrophages (AM) were obtained from 8 never smokers. AM viability, apoptosis, necrosis, and other markers were assessed	Flavourless liquids were used. E-cigs contained nicotine at 36 mg/ml	- Dose-dependent reduction in cell viability - Increased ROS production - Increased secretion of IL-6, TNF α, CXCL-8, monocyte chemoattractant protein 1 and matrix metalloproteinase 9	[84]
	Human	Korea National Health and Nutrition Examination Survey (47217 participants, 2013–2018). Lung function tests and some urinary markers were measured and compared among former smokers, current smokers, dual smokers and never-smokers.	Self-reported data were used. The questionnaire included ever used an e-cig in their lifetime, used an e-cig in the past 30 days and who smoked more or less than 100 cigs in their lifetime	Urinary levels of cotinine and NNAL were higher in both dual smokers and current smokers than in former and never smokers	[89]
	Human	Behavioural Risk Factor Surveillance System, USA (705,159 participants, 2016–2017)	Self-reported information on e-cigs used. The questionnaire included whether they ever used an e-cig or other vaping product, even just once time and in a lifetime	E-cigs users had 75% higher odds of chronic bronchitis, emphysema, or chronic obstructive pulmonary disease than never e-cigs users	[90]
Cardiovascular system	Mice	Female BALB/c mice, divided into control, tobacco smoke and e-cig aerosol groups	Exposed for 8 weeks. E-cigs contained 0 or 12 mg/ml of nicotine, the main excipient was PG or VG	E-cigs aerosol displayed a decline in parenchymal lung function at both functional residual capacity and transrespiratory pressures	[69]
	Mice	A/J mice. Investigated nicotine and the effects of e-cigs on lungs	Exposed to aerosolised phosphate-buffered saline, nicotine-containing (18 mg/ml) or nicotine-free e-cigs solution, 1 h/d for 4 months.	Nicotine-containing e-cigs caused airway hyper-reactivity, enlargement of distal airspace, mucin, cytokine, and protease production	[88]
	Human	16 healthy seldom smokers used to a maximum of 10 cigs/month. Participants were divided into two exposed and non-exposed groups, crossover design. Blood samples were collected at baseline and 1, 4 and 24 h exposure	Every participant inhaled 10 puffs of E-cigs vapour for 10 min using an e-liquid of 12 mg/ml nicotine	Elevation of endothelial progenitor cells in 1 h following exposure to e-cigs vapour and persisted for 4 h	[93]
	Human	40 healthy subjects (20 smokers and 20 nonsmokers) were recruited in a crossover, single-blinded study. Blood samples were collected and several markers determined	All participants smoked conventional cigs. One week later, the same individuals smoked an e-cig with a mean nicotine content of 16 mg	Both types of cigs were associated with elevated levels of soluble NOX2-derived peptide and 8-iso-prostaglandin F₂α and reduced nitric oxide bioavailability, vitamin E levels, and FMD.	[94]
	Human	Human embryonic stem cells (hESCs). Investigated the impact of tobacco cigs and e-cigs on heart development	Tobacco smoke or e-cigs aerosol was applied to the media. 1 aerosolized e-cigs contained 16 mg nicotine/cartridge or the smoke produced from 22 traditional cigs	- Lower expression of transcription factors and various late-stage contractile proteins observed in e-cigs treated group - Combustible cig's effects on cardiac health are more severe and diverse than those of e-cigs	[96]

(continued on next page)

Table 1 (continued)

Organ system	Species (human/animal)	Study design/methods	Exposure	Effects	Reference
Hepatic system	Rat	H9c2 cardiomyoblast cells (ATCC CRL-1446). Designed to evaluate the cytotoxic effects of e-cig liquid	Tobacco cigarette with 0.8 mg nicotine. E-cigs vapour of 20 e-cigs liquid samples and a “base” liquid sample (PG and VG, with no nicotine or flavourings)	Some e-cig-treated samples showed cytotoxic effects on cultured cardiomyoblasts	[95]
	Human	HepG2 cells, cell culture study	Exposed to a mixture of flavouring chemicals	Decreased cell viability and increased cytotoxicity	[114]
	Human	NHANES database from 2015 to 2018 (178,300 respondents), USA.	A self-reported questionnaire was considered to determine liver disease, e-cigs use and conventional cigs smoking status	E-cigs users and dual smoking were associated with an increased prevalence of liver diseases compared to non-smokers	[115]
	Rats	32 Male Wistar rats divided into 4 groups (control, nicotine, e-cigs %, and e-cigs treated)	Control group: IP injection of physiological saline, Nicotine group: IP injection 0.5 mg of nicotine/kg of bw/day, e-cigs % group: e-liquid without nicotine (less than 10 ml), and e-cigs group: IP injection of e-liquid containing 0.5 mg of nicotine/kg of bw/day (less than 10 ml). Treated for 4 weeks	- ALT, AST, ALP and LDH activities were increased in e-liquid exposed group - Reduced activities of antioxidant enzymes such as glutathione-S-transferase, superoxide dismutase, and catalase and increased malondialdehyde content	[109]
	Mice	24 virgin female Balb/c mice. Livers and plasma from dams and offspring were assessed.	Exposed to e-vapour before mating, during gestation and lactation, without exposure to the offspring. Tobacco-flavoured e-liquid (18 mg/ml nicotine), twice daily for 6 weeks	- Promoted lipid peroxidation and oxidative stress - Nicotine-free e-vapor promoted metabolic changes and hepatic damage in both dams and offspring	[110]
	Mice	Male and female young and adult C57bl/6 N mice. Assessed the effects of e-cigs on glycerol and glucose homeostasis	Exposed acutely or chronically for two consecutive hours, 5 days/week, for 9 weeks.	- Acute exposure to glycerol aerosols increased circulating levels of glycerol in female mice - Increased hepatic triglyceride and phosphatidylcholine levels were observed in female mice - Increased pulmonary resistance was noticed in young male mice	[111]
Renal system	Mice	Adult male ApoE ^{−/−} mice on a C57BL/6. Effects of electronic nicotine delivery systems (ENDS) determined	Exposed to saline or ENDS with nicotine (2.4%) aerosol for 12 weeks	- Accumulation of triglyceride levels in the liver - Increased oxidative stress and apoptosis in the liver	[112]
	Human	Korea National Health and Nutritional Examination Survey (2016–2017) included 10,692 participants	Never, ever, and current e-cigs users. The questionnaire included lifetime or the past month e-cigs users	The prevalence of hyperuricemia was higher among current e-cig users	[119]
	Rats	32 adult male Wistar rats in 4 groups (control, e-cig liquid treated without nicotine, e-cig treated with nicotine and nicotine treated groups.	E-cig refill bottles with tobacco flavour, with 18 mg/ml of nicotine or without nicotine. Intraperitoneal injection of 0.5 mg of nicotine/kg of bw/day. Treated daily for 4 weeks	- Creatinine levels increased in nicotine treated group - Total protein and sulfhydryl content were increased whereas superoxide dismutase and catalase activities were decreased in the e-liquids-treated group	[129]
	Rats	24 adult male Wistar albino rats, divided into 3 groups (traditional cigs smoking, e-cigs smoking and a control group)	E-liquid mixture of 0.6 mg/ml nicotine in a 55/45 ratio of PG and VG for up to 8 weeks	Higher levels of plasma symmetric dimethylarginine (SDMA) observed in both traditional cigs and e-cigs groups	[131]
Oral system	Mice	C57BL/6 and CD-1 mice exposed to e-cigs vapour	Exposed to e-cigs vapour daily for 3–6 months	GFR was reduced by 20% in e-cig exposed group compared to the control group	[75]
	Human	U.K. National Health Service (2015–2018). 886 participants were included in the randomized trials	Nicotine-replacement products were given for 3 months, or e-cigs with one bottle of nicotine-contained e-liquid (18 mg/ml) for 4 weeks.	Mouth or throat irritation was observed more frequently among the e-cigs user group (65.3%, vs. 51.2% in the nicotine-replacement group)	[23]
	Human	Tobacco and Attitudes Beliefs Survey 2015, USA. 533 adult participants	Comparison of the relationship between 30-day e-cigs use and symptoms in adult cigs users	Bleeding during brushing, sores or ulcers in the mouth were observed among participants who used e-cigs in the past 30 days	[139]
	Human	47 e-cigs users (group-1) and 45 NS (group-2). Inflammatory markers (TNF- α and IL-1 β) assessed in peri-implant sulcular fluid	Group 1: current vapers who used e-cigs for at least the past year; Group 2: subjects who never used tobacco throughout their life	Increased rate of plaque, more bone loss, higher levels of inflammatory markers and increased volume of crevicular fluid observed among e-cigs users	[141]
	Human	Human teeth, <i>Streptococcus mutans</i> UA157 (ATCC) used in the experiment	E-liquid (20/80 PG/VG) with 10 mg/ml nicotine. <i>Streptococcus mutans</i> (UA159) exposed to aerosols on tooth enamel	- E-cigs aerosols increased microbial adhesion to enamel - Flavoured aerosols led to a two-fold increase in biofilm formation and decreased enamel hardness	[144]
Auditory system	Human	Human middle-ear epithelial cell lines (HMEECs). Assessed cytotoxicity of e-liquids with or without nicotine	Exposed to e-liquids (various concentrations) and subsequently incubated for 24 h	- Reduced cell viability - Increased cytotoxicity	[155]
	Human	6-year girl, case study	Ingestion of a bottle (7 ml) of e-liquid (1.2 mg/ml nicotine)	Bilateral sensorineural hearing loss	[151]

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Table 1 (continued)

Organ system	Species (human/animal)	Study design/methods	Exposure	Effects	Reference
Musculoskeletal system	Human	Human MG-63 and Saos-2 osteoblast-like cells. E-liquids of various flavours with or without nicotine.	Treated for 48 h with 0.004%–4.0% dilutions	- Decreased Cell viability in a dose-dependent manner - Upregulated mRNA expression - Increased collagen type I protein expression	[157]
	Human	Human tumour-derived osteoblast-like MG-63 cells	Exposed for 24 h or 48 h to various concentrations of unvaped e-liquid or aerosol	- Decreased cell viability in a dose-dependent manner - Increased ROS production	[158]
	Human	Pooled 2017–2018 data NHANES, USA. Participants (5569) were divided into never (4519) and ever (1050) users (former and current)	Selection criteria: during the past 30 days, how many days have they used e-cigs	A higher prevalence of self-reported fragility fractures was found among ever-users than never user	[160]
Gastrointestinal system	Human	In 2019, USA (Wisconsin and Illinois), 98 case patients	Reported use of e-cigs devices and related products in the 90 days before symptom onset	Gastrointestinal symptoms included nausea (66%), vomiting (61%), abdominal pain (34%), and diarrhoea (44%)	[164]
	Human	NHANES (2015–2016), USA, 944 participants	261 subjects used e-cigs for at least one day and 683 subjects never used e-cigs over 30 days duration	E-cigs users had a higher prevalence of diarrhoea or vomiting (10.73%) compared to those who never used e-cig (8.64%)	[165]
Reproductive system	Mice/human	Assessed the effects of e-cigs use on the gut barrier using murine models, transcriptomics, and murine and human gut-derived organoid models	Exposed daily to e-cig aerosols (1 h/day) at two-time points: 1 week and 3 months	Nicotine-free e-cigs enhanced inflammation and decreased tight junction protein expression	[163]
	Rats	24 Adult male Wistar rats were divided into 3 groups (control, e-liquid without nicotine and both e-liquid and nicotine-treated groups).	E-liquid with and without nicotine (0.5 mg/kg BW) was administered intraperitoneally for 4 weeks.	- E-liquid with or without nicotine leads to reduced sperm viability and density (reduction in testosterone levels and testicular dehydrogenase activity). - E-liquid induced oxidative stress, causing increased activity of superoxide dismutase, glutathione-S-transferase, and catalase.	[166]
	Rats	14 male Sprague-Dawley rats were divided into 2 groups (control and exposed).	Exposed daily for 3 h, consisted of 11 cycles of 2 puff followed by 20 min of recovery. Exposed to PG, VG and flavouring components	- Increased LDH activity - Reduced 3β-HSD, 17β-HSD, G6PDH and SDH activities - ROS found in exposed rat testes - Increased LOX-linked activity	[168]
	Rats	30 male Wistar rats divided into 6 groups (A1 and B1 group: e-liquid vapour and 7.2 mg per cage/24, B1 and B2 group: treated with smoke from 10 cigs, C1 and C2 group: treated with air and noise)	Exposed to 5 days/week for 6 weeks, dose 210 mg nicotine.	- Malformations of sperm morphology and reduced spermatogenesis - Provoked apoptosis of spermatocytes and spermatogonia - Increased apoptosis of spermatogonia and spermatocytes, and acceleration of degeneration of testes	[169]
	Mice	Pregnant C57BL/6 J mice. E-cig components: PG and VG	Exposed 5 times/week to e-cigs vapour or sham	- E-cig-exposed dams exhibited a major delay in the onset of the first litter. - Changes in the chemokine, integrin, and JAK signalling pathways - Female offspring exposed to e-cigs <i>in utero</i> exhibited weight loss at 8.5 months	[173]
	Rats	Timed-pregnant Sprague-Dawley rats. Divided into 3 groups (control, juice, and juice + nicotine)	A flow rate of 2.5 L/min, with a 1-second e-cig vapour puff of about 42 ml puff every 20 s	- Decreased blood flow in the maternal uterine and fetal umbilical circulation was observed in juice + nicotine group - Decreased fetal weight and crown-rump length were observed in juice + nicotine group	[176]
	Mice	48 adult female and male Wistar rats (9–10 weeks old). Two groups control and treated with e-cigs	Dams (until 20 days of lactation) were exposed to e-cigarettes aerosol for 1 h daily	- Reduction in serum leptin level and fat content of the milk compared to control dams - Altered milk composition. Altered hormonal and biochemical markers in dams and pups	[182]
Endocrine system	Rats	Dams were exposed to nicotine during lactation. Adrenal function and leptin content in adipocytes and muscle were assessed in offspring	Exposed to nicotine (6 mg/kg/day) or saline for 14 days	- Higher adrenal catecholamine content - Increased visceral adiposity in offspring - Altered leptin levels on muscle and adipocytes in offspring	[183]
	Human	Endothelial function was assessed in smokers by measuring FMD and FMC of the brachial artery and arterial stiffness was measured by PTT and PWV	Exposed prior and 15 min after the e-cigarette vaping	- Reduced FMD and PTT - Increased FMC and PWV - Results indicate induction of endothelial dysfunction, arterial stiffness, impaired shear rate and skin perfusion	[108]
	Adult mice	124 C57BL/6 and 27 Nox2 null mice. E-cigs liquid consisted of 50% PG, 50% VG	Mice were exposed to e-cigs vapour over 1, 3, or 5 days for 2 h/day	Increased production of ROS in the frontal cortex.	[108]

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Table 1 (continued)

Organ system	Species (human/animal)	Study design/methods	Exposure	Effects	Reference
Immune system	Mice	Dams (3 groups) were exposed during and after pregnancy to ambient air, e-cig aerosols with nicotine, or e-cig aerosols without nicotine. Offspring cognitive testing was done at 12 weeks of age	Mice in each group were placed in a 9 L chamber filled with e-cigarette aerosols twice daily for 30 min	- Down-regulation of NOS in the entire brain - Reduced anxiety, deficits in short-term memory, and hyperactivity in offspring. - Increased global DNA methylation was noticed in offspring brains after birth	[175]
	Mice	Pregnant C57BL/6 mice. E-cig effects (with or without nicotine) were investigated during gestation and after birth	Exposed to e-cigs aerosols during gestation (3 h/day; 5 days/week) either with nicotine (13–16 mg/ml) or without nicotine and following birth, both pups and dams were exposed to the same exposure condition	- Transcriptome alterations in both offspring sexes - Downstream adverse neurobiological outcomes	
	Human	Healthy individuals (age 18–49 yrs) donated venous blood, neutrophils and NK cells were isolated from blood	Isolated cells were exposed to 7 flavoured, nicotine-free e-liquids. Neutrophils and NK cells (density 1×10^5 per well) exposed to 1.0% and 0.25% e-liquids, respectively in cell culture medium up to 1 h	E-liquid containing cinnamaldehyde exhibited a large dose-dependent immunosuppressive effect	[195]
	Human	119 adult participants (39 never-smokers, 40 e-cigs smokers and 40 conventional cig smokers). Saliva samples were collected from study subjects.	Current smoker (10 cigs/day for at least 12 months), nonsmoker (never smoked), and e-cigarette user (never smoked and using 0.5–1 e-cig/day per day for the past 6 months)	- A significant alteration in beta-diversity in e-cigs users when compared with conventional cigs smokers or never-smokers - An elevated level of IL-6 and IL-1β were found among e-cigs users compared to non-users	[196]
	Mice	Female mice (inbred C57BL/6 and outbred CD1), 6–8 weeks of age	Exposed to e-cigs aerosols for 60 min/day for 1–6 months	- Changes in gene expression in the lungs - Large reductions in IgA and CD4 - Showed effects on ACE2 expression	[197]

COPD [90]. Although these studies indicated an association between e-cigs and the risk of COPD, the findings are limited due to the cross-sectional nature of the studies. Therefore, prospective studies are needed to determine such an association.

Furthermore, studies showed elevated levels of chemokines and inflammatory cytokines (IL-6, IL-8 and TNF α) following AMs exposure to e-cigs extract in vitro [84]. Exposure of human airway epithelial cells to e-cigs also showed increased secretion of pro-inflammatory cytokines such as IL-6 and IL-8 [8]. In cellular metabolism ROS are generally produced which involves maintaining the redox homeostasis in the cells under physiological conditions, however, increased production of ROS causes airway inflammation and oxidative stress. There is also evidence regarding the presence of free radicals in e-cigs vapour [9]. In addition to the presence of e-cigs vapour, ROS generation is also occurred by e-cigs-exposed AMs, resulting in further increased airways ROS exposure [84]. Furthermore, e-cigs vapour may also induce oxidative stress and inflammation in pulmonary endothelial cells and bronchial epithelial cells [91].

5.2. Cardiovascular system

There are some animal and human studies that demonstrated the cardiovascular effects of e-cigs. One human study investigated the short-term effects of vaping and smoking on left ventricular function [92]. In this study, echocardiogram examinations were performed in healthy heavy smokers before and after smoking 1 cig and e-cigs users before and after using the device for 7 min. No instant effects of e-cigs on left ventricular function were observed in the e-cigs user group compared to the smoking group [92]. In another study, healthy occasional smokers were divided into two groups non-exposed and exposed to 10 puffs of e-cigs vapour for 10 min and their blood samples were collected at baseline and 1, 4 and 24 h duration following exposure [93]. The results showed a significant elevation of endothelial progenitor cells in 1 h following exposure to e-cigs vapour which persisted for 4 h but backed to baseline by 24 h. These findings suggested that the magnitude of these effects was the same as observed after combustible cigs smoking.

Another study by Carnevale et al. compared the effects of e-cigs vs combustible cigs on endothelial function and oxidative stress in healthy smokers and nonsmokers individuals [94]. The authors noticed that both types of cigs were associated with increased oxidative stress (determined by 8-iso-prostaglandin F $_{2\alpha}$ and soluble NOX2-derived peptide), impaired antioxidant defense (determined by decreased vitamin E levels), and endothelium dysfunction (determined by decreased nitric oxide bioavailability and flow-mediated dilation) [94]. The smokers had a higher baseline level of these markers compared to non-smokers, although, e-cigs seemed to have a lesser impact than combustible cigs on levels of the soluble markers.

The impact of e-cigs on cardiovascular health has been investigated in animal studies. In a study, the relationship between cardiac cytotoxicity and e-cigs vapour of 20 e-cigs liquid samples and a “base” liquid sample (50% propylene glycol and 50% glycerol, with no nicotine or flavourings) has been examined on cultured rat myocardial cells [95]. Results of this study indicated that cigs smoke extract showed cytotoxicity at a concentration above 6.5%, while base extract did not show cytotoxicity at any concentrations. However, cell survival did not show an association with nicotine levels of e-cig liquids. The cytotoxic properties of e-cigs depend on their preparation process and components used in flavourings. Another in vitro study used evaluated the impact of conventional cigs and e-cigs on cardiac development in vitro (human embryonic stem cell) and in vivo (zebrafish embryo) models [96]. Exposure of zebrafish to nicotine alone showed minimal effects on cardiac function, while exposure to cigs smoke or e-cigs aerosol showed a greater cardiac defect. On the other hand exposure of human embryonic stem cells to e-cigs extract represented the significantly lower expression of transcription factors and various late-stage contractile proteins. Combustible cigs' effects on cardiac health are more severe and diverse than those of e-cigs. Some researchers have indicated that nicotine delivery is mainly related to the adverse effects of e-cigs, while others have hypothesized that the aerosol of e-cigs without nicotine may also pose these effects. In a study by Schweitzer et al., the adverse effects of only e-cigs aerosol have been observed on endothelial barrier function and the authors suggested that these effects may exert by other toxic agents

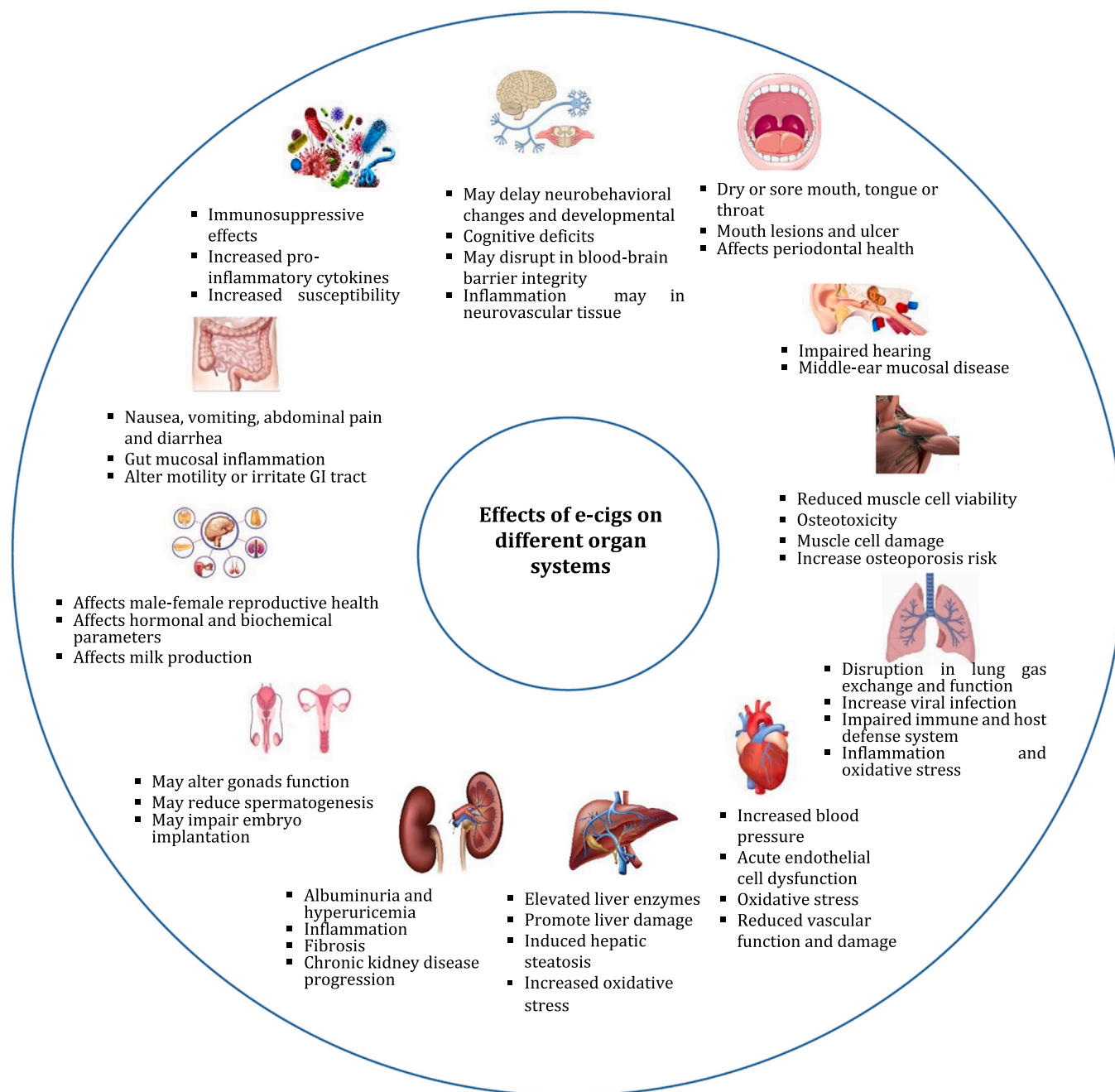


Fig. 2. Potential effects of e-cigs exposure on various organ systems in the human body.

such as acrolein [97]. These studies suggest that e-cigs vaping may pose some adverse effects on cardiovascular health, at least acute effects. These effects are varied based on the e-cigs' features and may be either less or similar to those of combustible cigs.

E-cigs use has been found to be linked with vascular function reduction and damage. For example, many studies have suggested that e-cigs use causes increased arterial stiffness in rodents and humans, as evidenced by elevation in pulse wave velocity and augmentation index [98,99]. Furthermore, e-cigs use affects endothelial nitric oxide synthase signalling pathways [100] which may be considered as the endothelial dysfunction biomarker in different vascular diseases including atherosclerosis and hypertension. The underlying mechanisms by which e-cigs exert these adverse effects have remained unknown. However, some studies indicated that e-cigs might cause ROS-mediated damage [101, 102]. In many studies, individuals who were e-cigs users either

occasionally or long-term were found to have altered serum or plasma markers connected to oxidative stress and cardiovascular disorders, including elevated levels of isoprostanes such as 8-iso-PGF_{2a}, myeloperoxidase, oxidant-producing enzyme, and decreased levels of non-enzymatic antioxidant vitamin E, and NO bioavailability [94,99, 103]. Studies also demonstrated the pro-thrombotic effect of non-nicotine constituents of e-cigs [104]. While increased platelet aggregation was observed in other studies after combined exposure to e-cigs vapour and nicotine [103,105]. Combined, these data indicate one possible mechanism whereby e-cigs exposure may contribute to myocardial infarction and stroke.

Consistent with the well-known sympathomimetic effects of nicotine, human exposure to e-cigarette aerosol may acutely increase blood pressure and heart rate [106]. Although many studies reported a positive relationship between e-cigs use and elevated heart rate and blood

pressure [98,106], there are also some inconsistent findings from a study that used short-term e-cigs exposure [107]. The hemodynamics effects of e-cigs are variable and may depend on the delivered nicotine concentration. While several studies demonstrated the effects of e-cigs on sympathetic induction, vascular pathology and platelet abnormalities [108], there is no information or little is known on the link between e-cig use and heart disease. Moreover, there are no specific reports on cardiac dysfunction as a result of e-cigs exposure in humans; however, chronic increases in blood pressure and arterial stiffness may potentially cause adverse cardiac remodelling. Unfortunately, this data is not available up to now but can be expected over time.

5.3. Hepatic system

The adverse effects of e-cigs on the lungs, and cardiovascular system are well presented; however, the detrimental effects of e-cigs on the hepatic system are not as well described. During smoking, nicotine is quickly absorbed by the lungs and metabolized in the liver, which may induce toxicity in the liver. A study in adult male rats demonstrated that e-liquid was associated with the elevation of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase increase (LDH), and alkaline phosphatase (ALP) [109]. In this study, nicotine alone was found to promote oxidative stress and reduce histopathological disorders, whereas nicotine with e-liquid caused more histopathological injuries in rats [109]. Another study by Li et al., 2020, showed that nicotine-free e-vapour may promote metabolic changes and hepatic damage in both pregnant mice and their offspring [110]. However, both nicotine and e-vapour were not associated with liver damage but induced hepatic steatosis in the adult offspring [110]. Glycerol contained in e-cigs liquids has been found to affect the liver as well as the aspects of glucose and glycerol homeostasis in young mice [111]. Furthermore, nicotine in e-cigs was found to cause increased oxidative stress, accumulation of triglyceride levels in the liver and increased apoptosis in the liver in adult mice [112]. E-cigs exposure may lead to NAD⁺ deficiency which indicates a link between e-cigs-induced DNA damage and mitochondrial dysfunction in the liver [113].

Up to now, there is limited evidence on the adverse effects of e-cigs on liver cells in human studies. A decreased HepG2 cell viability was observed when HepG2 cells were exposed to flavouring chemicals of E-cigs [114]. The authors also demonstrated that repeated exposure increases cytotoxicity and frequent vaping can cause hepatotoxicity [114]. According to the Centers for Disease Control (CDC), liver injury may also occur from E-cigs. A cross-sectional study using the National Health and Nutrition Examination Survey (NHANES) database from 2015 to 2018, showed that e-cigs users and dual smoking were associated with an increased prevalence of a history of different liver diseases compared to non-smokers [115]. Most of the animal studies reviewed here used a high concentration of nicotine, therefore, future studies using e-cigs should also consider zero or low levels of nicotine to determine its actual adverse effects on liver cells. Furthermore, more human studies are needed to determine the long-term effects of e-cigs on the liver.

5.4. Renal system

Combustible cigs exposure can cause proteinuria, fibrosis, and the progression of chronic kidney disease [116]. However, there is limited information about the effects of e-cigs on renal health. A cohort study conducted in Canada and the US for up to 5 years demonstrated that cigs use was associated with higher proteinuria in both adolescents and young adults [117]. In this study, subjects who smoked cigs in the past year had significantly higher proteinuria compared to non-smokers. However, occasional e-cigs use was not associated with proteinuria [117]. Another study by Podzolkov et al. showed an increased prevalence of albuminuria among university students who used either traditional cigs and/or e-cigs, as compared to those who were non-smokers [118]. However, the major limitation of this study was the small sample

size. In the Korean National Health and Nutritional Examination Survey (KNHANES) that included data from 2016 to 2017, elevated levels of cotinine and uric acid were found among current e-cigs users compared to those who were ex-or never users of e-cigs [119]. Elevated levels of uric acid (hyperuricemia), have been associated with chronic kidney disease and other health consequences such as obesity, hypertension, diabetes, dyslipidemia, liver dysfunction and metabolic syndrome [120–126]. Hyperuricemia causes renal injury through oxidative stress, inflammation and deposition of collagen [127,128].

Some recent animal studies also focused on e-cigs vapour exposure and kidney injury. In an animal study, adult rats were intraperitoneally (IP) exposed to e-cig refill fluid for 4 weeks and increased levels of creatinine were found in the e-cig treated group compared to the control group and modest changes in these renal function markers showed a positive association with oxidative stress [129]. In another rodent study, elevated levels of inflammatory proteins, such as angiopoietin-1 (Ang-1), leukaemia inhibitory factor (LIF), leptin, and epidermal growth factor (EGF), along with decreased levels of matrix metalloproteinase-3 (MMP-3) and chitinase 3-like 1 (YKL-40) were found in nicotine-containing e-cigs exposed mice group compared to the non-exposed control group [75]. In the same study, the glomerular filtration rate (GFR) was reduced by 20% in e-cig exposed group compared to the control group [75]. The associated molecular changes cause extracellular matrix and collagen production and ultimately lead to renal fibrosis and chronic kidney disease [130].

Long-term use of both traditional cigs and e-cigs was found to be caused oxidative stress-mediated renal tissue damage and declined renal function in rats [131]. A study by Lechasseur et al. showed that e-cig liquid exposure altered the expression of circadian rhythm-associated genes, including, *nr1d2*, *arntl*, *hspa1b*, *per1*, and *hspa1a* [132]. Alteration in the aforementioned circadian genes affects urine and electrolyte excretion and blood flow which may lead to alterations in GFR [133]. According to these experimental studies' findings, e-cigs vapour exposure appears to create adverse effects on a kidney similar to those observed in conventional cigs smoking. Although, some features observed in animal models may not be observed in human smokers. Moreover, laboratory studies mainly focused on the nicotine of e-cigs, while the effects of other components of e-cigs on kidneys remain undefined. Mechanistic studies may provide insights into the process through which e-cigs exposure affects renal health. Comprehensive epidemiological studies are also needed on both the general population and the population group who has preexisting chronic diseases.

5.5. Oral system

The deleterious effects of combustible smoking on oral health are well described. The major impact of conventional cigs smoking is related to oral cancer [134] and periodontal disease [135] which causes losing teeth [136]. However, the impact of e-cigs use on oral health is little known. Studies demonstrated that e-cigs users displayed fewer adverse complaints but increased positive symptoms than combustible cigs users [137]. A study by Cho et al. showed frequent oral discomfort among e-cigs users compared to never users [7]. In another study, mouth irritation was frequently observed among e-cigs users compared to those using nicotine replacement therapy [23]. Other mouth symptoms included black tongue, oral mucosal lesions, and burns found among e-cigs users [138,139]. Frequent e-cigs users had bleeding during brushing teeth, sores or ulcers in the mouth, and more than one cold, than those who were not using e-cigs [139]. These findings were supported by another study in which e-cigs users reported headaches, mouth ulcers, sensitive teeth and cold symptoms [140].

A study by Al-Aali et al. showed an increased rate of plaque, more bone loss, higher levels of inflammatory markers and increased volume of crevicular fluid among e-cigs users [141]. Another study followed a cohort of e-cigs users over 6 months and found a significant deterioration in periodontal health [142]. Some studies also examined the effects

of e-cigs aerosol on tooth structure. A self-reported gingival pain and/or bleeding, tongue pain, and cracked or broken tooth were found among e-cigs users [7]. These observations were supported by another study, where e-cigs exposure was found to alter enamel colour and reduce luminosity [143]. There are also some indications that e-cigs may promote dental caries. A recent study showed that e-cigs aerosol increased *Streptococcus mutans* adhesion to enamel and initiated biofilm formation [144]. Indeed, enamel exposed to e-cigs flavoured aerosol showed reduced hardness compared to controls. The enamel demineralization by this bacteria was associated with a high concentration of esters present in e-cigs liquid. Because commercially available e-cigs liquid contains several additives including sugar substitutes, sucrose, and acid, interactions with teeth may differ from one to another liquid [145,146].

Throat irritation is less frequent in e-cigs users than those in conventional cigs users. However, one study by Hajek et al. found increased throat irritation among e-cigs users compared to those who were using different nicotine replacement therapies [23]. Subjects using e-cigs containing nicotine frequently reported cough and sore throat compared to those nicotine-free e-cigs users [147]. Although, some e-cigs users reported negative throat symptoms as being related to some specific e-liquid flavours such as cinnamon, citrus, sour, or custard [148,149]. Tonsillitis, tonsilloliths, and laryngitis were also indicated by e-cigs users [138,140]. In contrast, in a case study, an e-cigs user who turned from a non-smoker experienced a resolution of her tonsillitis and an improvement in her tonsilloliths after starting to use e-cigs [150]. Other throat effects of e-cigs use included throat itchiness, throbbing, choking sensation, swallowing difficulty, hoarseness, and burnt feeling [140, 148].

Although several studies indicated the possible effects of e-cigs on mouth and throat, the major limitations of these studies were that these effects were generally self-reported by the users, without counting other confounding variables. Further studies are needed to evaluate these observations and answer some questions, like to what extent e-cigs exposure causes mouth and throat symptoms among those who shifted from conventional smoking to e-cigs, and among those who were never used to smoking.

5.6. Auditory system

There are several reports that conventional cigs smoking may have an adverse effect on the auditory system [151–154]. However, little is known about the effects of e-cigs on the auditory system except for a few reports. A study that examined the effects of e-liquid on human middle-ear epithelial cell lines (HMEECs) suggested that e-liquid can induce cytotoxic effects on these cells and reduce their viability by 32–64% [155]. In the same study, increased cytotoxicity was observed when menthol and the fruit-flavoured e-liquid were used. Results also showed increased cell cytotoxicity and viability when nicotine was included. Similarly, increased cell cytotoxicity was observed with propylene glycol. These findings suggest that e-cigs liquid may have a potential role in middle-ear mucosal disease. However, there is a lack of in vivo clinical evidence in humans for propylene glycol. Moreover, the clinical implications of this study were unclear, as possible confounding effects were not considered as observed in conventional cigs studies on ear disease. In the younger paediatric group, there is a concern over accidental ingestion of e-liquids. In a case study of a 6-year girl ingestion of a bottle (7 ml) of e-liquid (1.2 mg/ml nicotine) showed to cause bilateral sensorineural hearing loss [151]. The authors assumed that the hearing loss was related to the ingestion of e-liquid. The limitation of this case report was that it was subjective evidence of sudden hearing loss without pre-exposure history, therefore other possible causes cannot be ruled out.

5.7. Musculoskeletal system

The effects of combustible cigs smoking on the musculoskeletal

system have been described in many studies. Conventional cigs smoking can cause adverse effects on muscles, cartilage, tendons, and ligaments [156]. Moreover, a study focused on joints showed an association between conventional cigs smoking and increased joint disease progression, poor therapeutic response poor functional outcomes [156]. In contrast, there are few reports about the impact of e-cigs on musculoskeletal health. A study by Otero et al. examined the effects of e-liquid on bone-forming osteoblasts [157]. In this study, human MG-63 and Saos-2 osteoblast-like cells were treated with e-liquids flavours with or without nicotine. The results indicated that all e-liquids were associated with decreased cell viability in a dose-dependent manner and these effects were more pronounced for cinnamon-flavoured e-liquids independent of nicotine [157]. In addition, fruit-flavoured Mango Blast e-liquid (with or without nicotine) treated cells showed elevated expression of collagen type I protein compared to the culture medium alone. This study's findings suggest that flavoured e-liquid may cause osteotoxicity independent of nicotine. Another study investigated the effects of cinnamon-flavoured e-liquid (without nicotine) on bone-forming osteoblasts using human MG-63 cells [158]. Similar to previous study findings, a decreased cell viability was observed in a dose-dependent manner. In addition, increased ROS production was noted. Both cell viability and ROS production were more pronounced with cinnamon-flavoured e-liquids. However, no significant changes were found for collagen type I protein following the e-liquid exposure. The results of this study demonstrated that osteoblast-like cells are responsive to both e-liquids and aerosol particles and cinnamon-flavoured e-liquids mediated cytotoxicity may be associated with oxidative stress rather than alteration in the expression of collagen type I protein [158]. Thus, a number of researchers indicate that flavored vape have potential cytotoxic effects on bone cells which are independent of nicotine, signifying that flavoring agents alone may cause cellular damage [159].

A recent study using pooled 2017–2018 data from the NHANES showed a significantly higher prevalence of self-reported fragility fracture in e-cigs users than non-users [160]. Studies also suggested that besides the toxic effects of e-cigs on osteoblast cells, the long-term use of e-cigs may reduce mineral density in bone and increase the risk of developing osteoporosis [161]. Apart from osteoporosis, it has also been demonstrated that toxic metals in vaping may also influence other musculoskeletal diseases such as osteoarthritis and rheumatoid arthritis [162]. As limited information is available up to now, further research including longitudinal studies is required to explore the potential effects of e-cigs use on musculoskeletal health.

5.8. Gastrointestinal system

There is some evidence that e-cigs exposure may affect gastrointestinal tract (GI tract) functions. An animal study by Sharma et al. assessed the effects of e-cigs on the gut barrier using a combination of murine models, transcriptomics, and murine and human gut-derived organoid systems [163]. Histologic and transcriptome examinations indicated that chronic exposure to nicotine-free e-cigs enhanced inflammation and decreased tight junction protein (TJ) expression. As the gut mucosal barrier plays an important role in the host defense system, gut mucosal inflammation may lead to several chronic diseases [163].

E-cigs-induced GI symptoms have been reported in epidemiological studies. For example, a study conducted on 98 case patients from Illinois and Wisconsin, USA, reported gastrointestinal symptoms including nausea (66%), vomiting (61%), abdominal pain (34%), and diarrhoea (44%) [164]. A recent retrospective study examined the effects of e-cigs on the GI tract using data from the NHANES of 944 participants from 2015 to 16, of which 261 subjects used e-cigs for at least one day and 683 subjects never used e-cigs over 30 days duration [165]. The results showed that e-cigs users had a slightly higher prevalence of stomach or intestinal illness manifesting as diarrhoea or vomiting (10.73%) compared to those who never used e-cig (8.64%). These study findings suggest that e-cigs exposure may alter the motility or irritate the GI tract.

However, further studies are needed to determine the causal relationship and elucidate the mechanism by which e-cigs affect the GI tract.

5.9. Reproductive system

There is also evidence that e-cigs may also affect reproductive health. An initial study conducted by Golli et al. [166], examined the effects of e-liquid in an animal model. In this study, adult male Wistar rats were exposed to e-liquid either with nicotine or without nicotine (0.5 mg/kg BW) for 4 weeks. The results indicated that e-liquid regardless of with or without nicotine leads to reduced sperm viability and density, such as a reduction in testosterone levels and testicular dehydrogenase activity. In this study, reduced circulating testosterone levels were related to a decrease in the mRNA expression of two key steroidogenesis enzymes, 17 β -Hydroxysteroid dehydrogenases (17 β -HSD), and cytochrome P450scc. In addition, e-liquid induced oxidative stress, causing increased activity of antioxidant enzymes such as superoxide dismutase, glutathione-S-transferase, and catalase. Furthermore, in a histopathological examination, an alteration was observed in testis tissue [166]. In a continuation of the study in male rats, e-liquid exposure with nicotine or without nicotine showed a remarkable decrease in circulating levels of testosterone [167]. The study also showed an impact of e-liquid on oxidative status in the epididymides of the male rats.

A similar study also assessed the effects of e-liquid (which contained PG and VG but not nicotine) on rats' testicular functions [168]. Chemical analysis showed the presence of carbonyls such as formaldehyde, acetaldehyde, and acrolein in the vapor. Following e-liquid exposure, inhibition was observed in the activity of 3 β -HSD and 17 β -HSD, key enzymes in steroid synthesis. As a consequence, testicular marker enzymes such as glucose-6-phosphate dehydrogenase (G6PDH) and sorbitol dehydrogenase (SDH) functions were remarkably reduced, while the level of lactate dehydrogenase (LDH), a marker of cell damage was slightly elevated. Furthermore, the presence of ROS in exposed rat testes was noted, together with elevated levels in lipid peroxidation products and protein carbonyl formation, an alteration was also observed in detoxifying and antioxidant enzyme system. Although the testis' morphology was not examined, the results suggest an alteration in the structure and cellular organization of the testes. A higher rate of DNA unwinding in the WBC line and increased lipoxygenase (LOX)-linked activity were also found in testes examination. Taken together, the authors theorized that e-cigs vapours disrupt the enzymes involved in steroidogenesis and lead to impaired reproductive function in male rats and if the results are extrapolated to humans, e-cigs vapour exposure might alter gonads function in male vapers [168].

In another study, both conventional cigs smoke and e-cigs vapour exposure showed an effect on the morphology and function in the seminiferous epithelium, like vacuolization, reduced spermatogenesis, and provoked apoptosis of spermatocytes and spermatogonia and minor changes in sperm morphology [169]. Up to now, there are no specific reports on the effects of e-cigs use on spermatozoa. However, in a study, males' spermatozoa exposed to cinnamon and bubble-gum flavours added into the medium, showed a reduction in spermatozoa motility, causing slow movement of the cells [170]. In another part of this study, the authors exposed mice to cinnamon flavour bubble gum and noticed the damage in the germ cells of the mice testes related to especially bubble gum flavour. This study concluded that cinnamon flavour (also used in e-cigs) may affect the motility of spermatozoa, whereas the favour of bubble gum may damage testes cells which are generally associated with sperm production [170].

The impact of conventional cigarette smoking on the female reproductive system is well-defined [171,172]. However, the effects of e-cigs exposure on female reproductive health are not yet well documented, and the available data are mainly from investigations of animal models. A study using pregnant C57BL/6 J mice examined the effects of e-cigs exposure on implantation and offspring health [173]. In this study, pregnant mice were exposed to e-cigs vapour five times a week and

continued for four months. The results showed that dams exposed to e-cigs vapour exhibited a significant delay in the onset of their first litter compared to the non-exposed control group. Exposure to new dams in the initial stage of pregnancy impaired embryo implantation. Significant changes in the chemokine, integrin, and JAK signalling pathways were also observed when RNA microarray analysis was performed in pseudopregnant mice from day 4.5. Moreover, at 8.5 months, significant weight was noted among female offspring who were exposed to e-cigs in utero. This study findings suggest that e-cigs exposure in mice may impair pregnancy initiation and fetal health. Some studies also shown that e-cigs vapor exposure during pregnancy may alter offspring's behavior and cognition [174,175].

The effects of chronic e-cigs aerosols exposure on early development were determined in the Sprague-Dawley rat model [176]. The rats were grouped as Pair-Fed Juice, Juice+Nicotine groups, and Pair-Fed Control, and then exposed to prenatal or prenatal+postnatal paradigm in a vaping system. The Juice+Nicotine exposure group showed a significant decrease in fetal weight and crown-rump length. Pre- and postnatal exposure to Juice+Nicotine caused a decrease in pup weight on a postnatal day. In the Juice+Nicotine group, decreased blood flow was observed in the maternal uterine and fetal umbilical circulation. This study results indicate that chronic exposure to e-cig aerosols containing nicotine may pose adverse developmental effects in early life. A study by Chen et al. using a mice model showed that e-cigs exposure during pregnancy and lactation affected the lung health of both pups and dams with remarkable changes in DNA methylation [177]. All the animal studies that assessed the effects of e-cigs exposure on reproductive health demonstrated that early or postnatal exposure to e-cigs vapours containing nicotine caused adverse effects, including reduced length and weight. So far, up to now, no study assessed the reproductive effects of e-cigs during the pregnancy period in women. However, chronic exposure to e-cigs can provide a similar, even higher level of nicotine in the circulation system as provided by conventional cigs smoking [178]. Literature reviews already indicated that e-cigs use in women's pregnancy are not safe for fetus [179,180]. As the impact of e-cigs on human development is not well known, promoting awareness among healthcare providers and general people of the benefits and risks of e-cigs use during pregnancy period.

5.10. Endocrine system

The impact of combustible cigs on the endocrine system is well described. A literature review that included both animal and human studies indicated that conventional cigs smoking can affect hormonal levels and metabolic homeostasis [181]. Several experimental studies have assessed nicotine exposure effects during the lactation period, and it has been shown that both short- and long-term exposure to nicotine affects neonatal and child development [182]. It is evident that conventional cigs smoke can disrupt the endocrine system, leading to hypothyroidism, increased visceral adiposity, and altered leptin levels on muscle and adipocytes [183], thus causing weight gain in adulthood [184,185]. Epigenetics studies have also suggested a possible effect of nicotine exposure on DNA methylation and cortisol levels [186].

In contrast to conventional cigs, little is known about the endocrine effects of e-cigs exposure. A very recent animal study investigated the effects of e-cigs aerosol exposure on dams and pups [182]. In this study, lactating dams were exposed to e-cigs aerosol (day 4–21) or received fresh air (control) during lactation (day 4–21). The results showed that maternal exposure to e-cigs aerosol during lactation caused a significant reduction in serum leptin level and fat content of the milk compared to control dams. Furthermore, e-cigs aerosol exposure altered the milk composition, as well as hormonal and biochemical markers in both dams and pups. This study findings suggest that e-cigs exposure during lactation may affect milk production and hormonal and biochemical parameters in dams and pups. The authors hypothesized that a similar event might occur in breastfeeding mothers and their infants if the

nursing mothers are exposed to e-cigs during lactation. Several animal studies also demonstrated that e-cigs exposure can affect male and female reproductive health, as described in the section on the effects of e-cigs exposure on the “reproductive system”.

5.11. Nervous system

Despite increasing awareness of the adverse effects of e-cigs usage, little is known about their impact on the nervous system. Nicotine is known to cause a disruption in brain development, interference with cognitive function, and lead to several mental and physical health problems. A recent study supported by the National Institute on Drug Abuse showed that e-cigs-related changes in the brain are close to those caused by nicotine from other sources, such as conventional cigs or nicotine lozenges [187]. However, whether e-cigs use has the same impact as conventional cigs remains unclear. To date, there is limited information regarding the neurotoxicity of e-cigs in humans. However, a few animal studies have demonstrated the neurotoxicity of e-cigs, especially during neurodevelopment. In a human study, e-cigs vapour exposure was shown to cause reduced flow-mediated dilation (FMD) and pulse transit time (PTT) and increased low flow-mediated constriction (FMC), and Pulse wave velocity (PWV), indicating endothelial dysfunction, arterial stiffness, impaired shear rate and skin perfusion [108]. In the same study, exposure to nicotine-free e-cigs vapour in adult C57BL/6 mice showed to cause increased production of ROS in the frontal cortex and down-regulation (mRNA and protein) of the neuronal nitric oxide synthase (NOS) in the entire brain [108].

In an animal study, dams were exposed to e-cigs aerosols with or without nicotine to examine the offspring's cognitive performance [175]. The results showed a decrease in anxiety and short-term memory, and hyperactivity in offspring following maternal exposure to e-cigs. Exposure to e-cigs without nicotine was found to cause increased global DNA methylation immediately after birth, affected histone acetyltransferase and promoted alteration within genes associated with neuronal functions [175]. Another study indicated that even switching from conventional cigs to e-cigs during pregnancy may also cause neurological damage in the offspring [188]. Epigenetic changes were also found in offspring of C57BL/6 mice dams exposed to e-cigs aerosols with or without nicotine during pregnancy and lactation [174]. E-cigs with nicotine exposure are also found to cause oxidative stress, disrupt blood-brain barrier integrity, inflammation in neurovascular tissue, and exacerbate post-ischemic brain injury at a similar rate found to conventional cigs exposure [189], which indicates that e-cigs use might increase the risk of brain injury and stroke.

Besides e-cigs vapour, e-cigs-liquid exposure was also found to have adverse effects on neuro activity. Altered cognitive functions were observed when adult rats were exposed to e-liquid for four weeks, and the alteration was more pronounced in the nicotine-free group [190]. E-cigs neurotoxicity may be related to alteration in mitochondrial membrane potential and mitochondrial dynamics which leads to mitochondrial hyperfusion and swelling [191]. Taken together, e-cigs aerosol and liquids may cause neurobehavioral changes, developmental delays, and cognitive deficits. Moreover, e-cigs aerosols contain several additional components, such as acrolein, and formaldehyde [192,193], and various trace elements, such as phenolic compounds, heavy metals, hydrocarbons, and other detrimental chemicals. These additional substances may also affect neural activity as reviewed by Ruszkiewicz et al. [194]. Although available data indicated the adverse effects of e-cigs exposure on the nervous system, further research is required to determine its specific effects on the brain.

5.12. Immune system

While conventional cigs have been shown to affect immune cell function, the effects of e-cigs on immune cell function are not well understood. A group of researchers found that e-cigs exposure can affect

the human immune system [195]. This study evaluated the effects of nicotine-free e-liquid on primary human alveolar macrophages, natural killer (NK) cells and neutrophils. Cells were exposed to different e-liquids and their functional responses to pathogenic stimuli were determined. Results showed that e-liquid containing cinnamaldehyde exhibited a large dose-dependent immunosuppressive effect. The authors concluded that cinnamaldehyde can potentially impair respiratory immune cell function [195]. A study by Pushalkar et al. examined the saliva of 119 participants for alteration in the oral microbiome and found a significant alteration in beta-diversity in e-cigs users when compared with conventional cigs smokers or never smokers [196]. In a further analysis of host immune response, an elevated level of interleukin 6 (IL-6) and IL-1 β were found among e-cigs users compared to non-users. When epithelial cells were exposed to e-cigs aerosols showed increased susceptibility to infection. In Vitro exposure of premalignant Leuk-1 and malignant cell lines to e-cigs aerosol and challenged by oral pathogens showed increased inflammatory response [196]. This study findings suggest that e-cigs users are more susceptible to infection. Another study in an animal model demonstrated that inflammatory pathways from both innate and adaptive responses may be affected by the chronic use of e-cigs aerosols [197]. In a further recent study, exposure of oral epithelial cells to e-cig aerosols showed immunosuppression, as measured by a reduction in the levels of IL1 β , IL6, and IL8 by the epithelial cells during co-culture with *S. aureus* [198]. Furthermore, the study also showed overexpression of the inflammatory regulator COX2. The authors theorize that e-cigs enhance *S. aureus* colonization and regulate the oral inflammatory response, likely by promoting oral periodontitis and preneoplasia [198].

5.13. Developmental effects

The developmental effects of e-cigs exposure have also been reported by a few studies. In a study, the growth and osteogenic differentiation of bone marrow-derived mesenchymal stem cells (MSCs) were evaluated after exposure to smoke extracts [199]. Data revealed that both conventional cigs and e-cigs exposure reduced proliferation and altered the morphology of MSCs. Moreover, the levels of alkaline phosphatase (an osteogenic marker) were reduced and the process of osteoblastic differentiation was interrupted. Another study examined the effects of e-cigs vapour on placental trophoblast function using HTR-8/SVneo cells. The results showed that e-cigs-conditioned media significantly reduced the invasion of placental trophoblast and tube formation [200]. In a frog model, with or without nicotine contained e-cigs flavour exposure in embryonic development was found to cause craniofacial defects [201]. It has also been suggested that maternal exposure to e-cigs may increase mRNA expression of brain neuropeptide Y and inducible nitric oxide synthase in offspring [202] and may cause short-term memory impairment and hyperactivity [175]. Exposure of pregnant mice to nicotine-free e-cigs showed increased accumulation of body fat in offspring, suggesting that utero exposure may cause metabolic abnormalities in the offspring [202]. Recent animal studies further demonstrated that exposure to e-cigs during pre-conception and prenatal stages may impair embryo implantation and the metabolic health of offspring in later life [173]. Fetal exposure to nicotine during pregnancy is a public health concern and the effects of e-cigs on fetal development need to be established.

5.14. Metabolic effects

The metabolic effects of conventional cigs smoking have already been established in many studies. However, limited data is available about the link between e-cigs exposure and metabolic disorders. A study determined the effects of nicotine-containing e-cigs vapour in mice models where male Balb/c mice were given a high-fat diet (HFD) over 16 weeks and exposed to e-vapour over the last 6 weeks [203]. The results indicated that e-vapour decreased fat mass in HFD-fed mice, and

e-vapour contained nicotine increased glucose tolerance. Whereas, e-vapor increased lipid levels in both the liver and blood. A study in Korea included a large number of human participants to determine the relationship between e-cig exposure and metabolic syndrome [204]. In data analysis, current e-cigs exposure showed a significant association with the increased risk of metabolic syndrome. Both conventional cigs and e-cigs exposure was associated with central or abdominal obesity [204]. A recent review indicated that e-cigs exposure does not affect the insulin resistance process, but can influence blood glucose levels and the development of pre-diabetic conditions [205]. E-cigs exposure was also found to affect the lipid profile, especially TG levels. In most cases, e-cigs use was associated with the risk of developing abdominal obesity [204]. Future studies can reveal how e-cigs exposure leads to metabolic abnormalities.

5.15. Other effects

E-cigs exposure has also been found to be associated with sleep health. A study by Brett et al. reported that similar to conventional cigs, e-cigs were associated with worse sleep health in young adults [206]. Another study showed a significant association between e-cigs use and higher odds of sleep-related problems in adolescents [207]. In addition, e-cigs exposure was found to cause dizziness or lightheadedness (27.1%) in a cross-sectional study in the USA [208]. Furthermore, e-cigs exposure may also cause skin allergy and eye irritation due to propylene glycol present in e-liquid [209,210]. In a study in rats model, both medium and high-nicotine-content e-cigs exposure showed flap necrosis and hypoxia when compared to the conventional cigs exposure group [211].

6. Toxicity mechanisms of e-cigs

Although the adverse effects of e-cigs have been investigated in numerous studies, the underlying mechanisms of toxicity remain obscure. Early studies demonstrated that the adverse effects of e-cigs are mainly related to oxidative stress, inflammation and DNA damage. The main toxicity mechanisms of e-cigs are described as follows. The humectants agents present in the e-liquid can generate the carbonyl molecules such as acetaldehyde, formaldehyde, and acrolein upon thermal degradation [49], and exposure to such chemicals may induce adverse health effects [212]. The inhaled acrolein from the e-cigs vapour increases vascular permeability due to the activation of (transient receptor potential ankyrin 1 (TRPA1), a sensory receptor primarily expressed on the surface of certain cells [213]. The TRPA1 activation signals the Ca^{2+} -dependent release of neuropeptides and leads to bronchoconstriction [214] (Andre E et al., 2008). Also, inhaled acrolein can cross the alveolar-capillary barrier [215] and diffuse across the cellular membrane due to its amphiphilic nature and covalently modify the cytoplasmic proteins [216]. Additionally, acrolein may inhibit protease enzymes and cellular detoxification enzyme glutathione S-transferase and generates oxidative stress and inflammation [217,218].

Exposure to nicotine-containing e-cigs can stimulate protein kinase C alpha (PKC α) and extracellular signal-regulated kinase (ERK) signalling pathway through the receptors such as $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7nAChR$) [219], leading to the secretion of inflammatory marker IL-6 [88]. IL-6 may further cause an inflammatory response through Janus kinase/signal transducers and activators of the transcription (JAK/STAT) pathway [88]. IL-6 may also lead to the excessive activation of inflammatory cells and macrophages [220], triggering lung injury mediated by NK cells [221]. Nicotine-containing e-cigs can promote ROS-mediated autophagy impairment [222]. Furthermore, e-cigs exposure triggers neutrophil inflammatory response through the release of Interleukin 8/chemokine (C-X-C motif) ligand 8 (IL-8/CXCL8) (neutrophil chemoattractant) and matrix metalloproteinase 9 (MMP-9) [223]. MMP-9 and neutrophil elastase (NE) activity is associated with COPD severity and related to neutrophilic inflammation [224]. E-cigs

aerosol exposure can increase mitochondrial ROS production and NADPH quinone oxidoreductase 1 (NQO1) expression [225]. NQO1 is associated with activating the Nrf2 pathway [225]. Similarly, exposure of primary human bronchial epithelial (NHBE) cells to e-liquid resulted in the induction of oxidative stress-response genes suggesting that e-cigs can stimulate the Nrf2 signalling pathway via oxidative stress [226].

Exposure to e-cigs aerosol is also associated with DNA damage, assumed to take place through a conversation of nicotine to nitrosamines in situ metabolism [227]. It has been indicated that e-cigs aerosol-induced mitochondrial ROS production may cause fragmentation of nuclear DNA in HFL-1 cells [228]. Further studies showed that acrolein induced increased ROS production and GSH depletion and formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) in HepG2 cells and leads to DNA damage [217]. In addition to the above 3 mechanisms, some studies suggested new toxicity mechanisms that involved cell apoptosis, epithelial-mesenchymal transition (EMT), and transcriptomic changes. After lung inhalation of e-cigs into the blood circulation, it can still cause adverse effects on other organ systems and may induce different physiological mechanisms. Thus, further research data are required to get deep insights into the toxicity mechanisms of e-cigs. Furthermore, it is also important to understand the differences in the toxicity mechanisms between traditional cigs and e-cigs.

7. Exposure risk for non-users

E-cigs use may also cause exposure risks for non-users but to a lesser extent than combustible cigs. Refilling e-cigs cartridges may contain nicotine at a toxic level and children at particularly at high risk of such refilling [46]. Nicotine from e-cig aerosol may remain on solid surfaces for weeks to months and can react with ambient nitrous acid to produce tobacco-specific nitrosamines which are carcinogenic compounds [229, 230]. Moreover, persistent nicotine residuals on indoor surfaces may lead to an increased risk of exposure through inhalation, skin, and ingestion [229]. A study by Schober et al. assessed the indoor air quality after using e-cigs of nine subjects for two hours duration [231]. The results showed an increased number of suspended particles consisting of glycerin, nicotine, and 1,2-propanediol. Although e-cig flavours may increase their appeal to children, a high level of nicotine in e-liquid can be life-threatening [46]. The refilling solution has been shown to cause adverse effects on embryonic cells [159]. However, currently, there is limited information about the potential risks of e-cigs exposure to the fetus.

8. Knowledge gaps and recommendations for future research

While most of the studies reviewed here indicated the adverse effects of nicotine in e-cigs in various organ systems, the potential impact of other components of e-cigs and the heated aerosol mixture on the organ system remains largely undefined. Another research gap is the lack of or limited mechanistic studies required to improve understanding of the pathways through which e-cigs exposure leads to adverse effects on multiple-organ health. Inflammation and toxicity may shift from one organ to another organ. However, it remains unknown whether similar events may occur for e-cigs exposure. In addition, there is a lack of studies regarding the second-and-third hand exposure to e-cigs by measuring indoor air and biomarkers of human exposure. Moreover, it is not clear yet about the effects of long-term e-cigs use among those with pre-existing chronic diseases such as kidney disease, diabetes, and hypertension, as well as the sex and age-dependent effects of long-term exposure to e-cigs.

Vitamin E- acetate has been banned and removed from e-liquids as a component after investigating its association with the outbreak of EVALI. Furthermore, using THC in e-cigs is another rising concern as it influences adolescents and younger people to try e-cig to feel better and reduce anxiety. Evidence about the adverse effects of e-cigs on the respiratory system and other organ systems are constantly emerging from

in vitro model, animal studies and human studies; however, its manufacturing companies are growing rapidly. Therefore, there is a need for regulatory studies to develop an aerosol system that will be followed by manufacturing companies. Moreover, the chemical composition and chemical combination of e-cigs should be defined. A major portion of the studies focused on the toxicity of a single component of e-cigs. Therefore, it is necessary to determine the combined effects of e-cigs components. The use of e-cigs in the young generation is increasing at an alarming rate, therefore, there is a need for guidelines on who can legally buy e-cigs and also there is a need to define the effects of e-cigs exposure on the adolescent organ systems. As e-cigs use has evolved in the last two decades, there is a lack of long-term studies to determine the effects of e-cigs on various organ systems. Thus, the chronic effects of e-cigs exposure need to be addressed in future studies. Large-scale longitudinal and prospective studies are needed to understand the implication of e-cigs use in those with pre-existing medical conditions including chronic respiratory diseases. To further these investigations, organ-specific toxicological and functional studies are needed. Furthermore, a public health risk assessment needs to be done considering the duration of e-cigs use, prevalence of illness, hospital visits and admission, and deaths related to e-cigs exposure.

In addition, the following questions need to be addressed, such as how can we make e-cigs safer? It is important to identify specific components/flavours and improve the heating technology. Moreover, needs to consider the different jurisdictions and also the interactions between tobacco control and vaping in countries where the tobacco industry is less restricted. Then, what are the consequences of long-term use (>1 year) in people who have switched (less than smoking but not zero)? What are the consequences in children (short-term effects on lung growth and longer-term risk of “lifetime” use) who might not otherwise have smoked (though most children who vape have or still smoked)? How to make them less appealing to children who have not smoked, while still available as a stop-smoking aid? It is also important to investigate whether taking some devices through the medicine licensing process may solve many of the concerns. Furthermore, for non-nicotine vaping – it is needed to make a clear distinction between legal/licensed products and these.

9. Conclusions

The adverse effects of conventional cigs on human health have been well described. In contrast, the impact of e-cigs use on various organ systems has not been well defined yet. A number of emerging studies showed the adverse effects of e-cigs exposure on the multiple-organ system. The differences in research methodology, the relatively small studies, the inconsistent findings between studies and inadequate long-term exposure data make it difficult to draw a firm conclusion about the safe use of e-cigs. Although the adverse effects of e-cigs exposure have been reported in several studies, more specific prospective and mechanistic studies are needed to improve our understanding of its potential health effects. Compared to combustible cigs, e-cigs cause fewer adverse effects on health, but data on long-term exposure is lacking. In addition, a wide variation in e-cigs products and a lack of standardized testing methods make it challenging to evaluate the existing data. E-cigs may be a useful tool for smoking cessation, but well-supportive data are missing. Furthermore, the vaping industry is rapidly increasing worldwide, therefore, the standard of the products including ingredients criteria, chemical composition and manufacturing quality need to be developed and monitored by regulatory bodies.

Environmental implications

In addition to human health effects, e-cigs can also have environmental effects. For instance, the disposal of single-use of plastic from e-cigs may increase plastic pollution. Furthermore, it can increase the tech waste from the vape parts including e-cigs batteries and their toxic

material which can progressively leach into the environment. Furthermore, e-cigs emitting particles may contribute secondhand aerosol to the environment during exhalation by the users.

CRediT authorship contribution statement

N.A., contributed to the conceptualization, data curation, writing and editing of the manuscript. J.X., M.E., and M.P., contributed to drafting some parts of the manuscript. J.B.S., contributed to the conceptualization, review and editing. All authors read and approve the final version of the manuscript.

Declaration of Competing Interest

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.

Data Availability

At the introduction of the review, we explain how we gathered the data and papers that is mentioned in the review. Data will be made available on request.

Acknowledgments

The author thanks Prof Nick Hopkinson for the helpful comments on primary version of the manuscript. J.B.S acknowledges support from Bill & Melinda Gates Foundation and BBSRC (Grants INV-016631 and BB/V019791/1, respectively); also from the Commonwealth (PhD Scholarship Split-site to Mr. Joseph Xavier), EPSRC CDT in Aerosol Science (PhD Scholarship to Mr. Melih Engur) and a pump-prime award from the Integrated Biological Imaging Network (IBIN), a Technology Touching Life MRC Network (MR/W024985/1).

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