



Review Article

Assessing the impact of e-cigarettes on human barrier systems: A systematic review

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ABSTRACT

The use of e-cigarettes has grown rapidly in recent years, raising concerns about their impact on human health, particularly on critical physiological barriers such as the blood-brain barrier (BBB), alveolar-capillary barrier, and vascular systems. This systematic review evaluates the current literature on the effects of e-cigarette exposure on these barrier systems. E-cigarettes, regardless of nicotine content, have been shown to induce oxidative stress, inflammation, and disruption of tight junction proteins, leading to impaired barrier function. Key findings include compromised pulmonary function, increased vascular stiffness, and neuroinflammation. The review highlights potential long-term health risks associated with e-cigarette use, such as cardiovascular disease, neurodevelopmental disorders, and multi-organ fibrosis, and emphasizes the need for public health interventions to regulate e-cigarette use, especially in vulnerable populations like pregnant women and adolescents.

Introduction

E-cigarettes are battery-powered devices that heat a liquid solution containing propylene glycol or glycerol, nicotine, and flavorings to produce an inhalable aerosol.^{1,2} They consist of an aerosol generator, flow sensor, battery, and liquid storage area.² Unlike traditional cigarettes, e-cigarettes do not contain tobacco or require combustion.¹ E-cigarettes are marketed as healthier alternatives to smoking and aids for smoking cessation.¹ However, their long-term health effects are largely unknown, and there are concerns about potential toxicant exposure and adverse health effects for both users and bystanders.³ While evidence suggests e-cigarettes may help smokers reduce or quit smoking,⁴ more research is needed to fully understand their efficacy and safety. Product variability and user modifications can affect nicotine delivery and potential risks.²

Electronic nicotine delivery systems (ENDS) or e-cigarettes have seen a rapid increase in awareness and use, particularly among youth and young adults.^{5–7} While marketed as a safer alternative to traditional cigarettes, the long-term health effects of ENDS remain largely

unknown, with some studies indicating potential risks.^{8,9} ENDS have evolved through multiple generations, offering increased complexity and personalization options.¹⁰ Their use has been associated with both potential benefits for smoking cessation and concerns about gateway effects to tobacco use.¹¹ The dramatic growth in ENDS sales has surpassed that of nicotine replacement therapy, with projections suggesting they may overtake traditional cigarette sales within a decade.¹² Given the rapid expansion of ENDS use and the uncertainties surrounding their impact, continued research and monitoring are essential.^{6,11}

E-cigarette use has become increasingly prevalent, especially among young adults and current or former smokers. Studies report varying rates of e-cigarette use, with ever-use ranging from 5.5 % to 56.6 % and current use from 0.2 % to 27 %.¹³ Prevalence is highest among those aged 18–24.^{14,15} Current smokers show the highest rates of e-cigarette use, followed by former smokers.^{13,16} Common reasons for e-cigarette use include curiosity, smoking cessation, and use in non-smoking areas.^{17,18} Factors associated with higher e-cigarette use include male sex, younger age, and smoking status.^{13,19} While e-cigarettes are often perceived as a safer alternative to conventional cigarettes, concerns

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remain about their safety and effectiveness as cessation aids.^{15,20}

The long-term health implications of e-cigarette use remain uncertain, but emerging evidence suggests potential risks. E-cigarette aerosols contain harmful substances, including carcinogens and irritants.^{8,21} Short-term use may lead to acute cardiopulmonary effects and oxidative stress.^{21,22} Animal studies indicate negative impacts on various organ systems, including cytotoxicity, inflammation, and emphysematous changes.^{21,23} Long-term exposure in mice has shown COPD-like pulmonary changes.²⁴ While e-cigarettes may be less harmful than traditional cigarettes, they are not risk-free.^{22,25} Concerns include potential cardiovascular disease risk and DNA damage.²⁴ The rapid evolution of e-cigarette devices and lack of long-term studies highlight the need for further research to fully understand their health impacts.^{23,26}

E-cigarette use has increased rapidly worldwide, with a global lifetime prevalence of 23 % and current use at 11 %.²⁷ While e-cigarettes are often marketed as a safer alternative to traditional cigarettes, emerging evidence suggests potential risks to various physiological systems.²³ Acute effects have been observed in pulmonary, cardiovascular, and immune systems, highlighting the need for long-term research.²³ E-cigarettes deliver nicotine and other toxicants, potentially posing risks for dependence and uptake of combustible cigarettes.^{23,28} Despite lower levels of known toxicants compared to traditional cigarettes, e-cigarettes may produce harmful effects through their own chemical constituents.²³ The efficacy of e-cigarettes for smoking cessation remains unestablished, and their potential benefits must be weighed against the risks.^{23,25} Further research is needed to fully understand the health consequences of e-cigarette use.²⁹

While potentially less harmful than traditional cigarettes, ENDS can disrupt lung lipid homeostasis and impair innate immunity, independent of nicotine content.³⁰ Chronic exposure to e-cigarette vapor may lead to systemic inflammation, multiorgan fibrosis, and cardiorenal dysfunction.³¹

E-cigarette use has raised concerns about its impact on various barrier systems in the body.

The formation of tissue barriers begins early in development, playing a crucial role in determining cell fate, differentiation, and the formation of organs. Maintaining these barriers, that protect the human body from harmful agents, is essential for proper organ function throughout adulthood and aging. When tissue barrier formation or function is impaired at any stage of life, it can lead to various disease conditions.³²

The Blood-Brain Barrier (BBB) is one of the most critical, maintaining brain homeostasis and protecting against blood-borne threats.³³ Other barriers, such as the blood-testis barrier,³⁴ skin,³⁵ mucosal surfaces,³⁶ placental barrier,³⁷ and gut barrier,³⁸ all have specialized roles in maintaining overall health and preventing disease. Together, these barriers form an integrated system that shields the body from external and internal threats.

It has been demonstrated that chronic e-cigarette use compromises the gut barrier, triggering inflammation and increasing susceptibility to infections.^{39,40} The airway barrier function is also affected, with e-cigarette vapor causing decreased viability and function of airway cells.^{31,41} Furthermore, e-cigarette exposure disrupts lung endothelial barrier function, leading to increased inflammation and oxidative stress.⁴² These effects are not solely attributed to nicotine, as non-nicotine components of e-cigarettes also contribute to barrier disruption and inflammation.^{39,43} The long-term health consequences of e-cigarette use, including potential respiratory and cardiovascular effects, warrant further investigation.⁴⁴

Despite their potential as smoking cessation aids, concerns persist about ENDS' long-term health effects and their appeal to young people.^{45,46}

Research indicates that e-cigarette vapor can disrupt airway epithelial barrier function, leading to increased permeability and inflammation. Chronic exposure to e-cigarette aerosols containing nicotine and flavorings has been shown to decrease trans-epithelial electrical resistance, impair ciliary function, and increase

inflammatory cytokine release in human bronchial epithelial cells.^{47–49} These effects were observed even at non-cytotoxic concentrations. Long-term inhalation studies in mice demonstrated systemic inflammation, multiorgan fibrosis, and cardiorenal dysfunction.³¹ E-cigarette exposure also disrupted blood-brain barrier integrity and promoted neuroinflammation in animal models.⁴³ The harmful effects were partly independent of nicotine content, with some flavoring chemicals like acetoin and maltol inducing significant inflammatory responses.⁴⁷ Overall, these findings suggest that e-cigarette use may have detrimental effects on respiratory and systemic health.^{42,48}

This systematic review aims to comprehensively evaluate the current literature on the effects of e-cigarette vapor on critical physiological barriers.

Material and methods

Study design and protocol

This review seeks to elucidate the extent to which e-cigarette use may disrupt these barriers, thereby affecting overall health and increasing susceptibility to disease. The review will focus on synthesizing data related to the blood-air barrier, blood-aqueous barrier, blood-brain barrier, blood-retinal barrier, blood-testis barrier, glomerular filtration barrier.

The Sample, Phenomenon of Interest, Design, Evaluation, and Research type (SPIDER) framework^{50,51} was applied to frame the research question of this systematic review and to support in defining the eligibility criteria. The SPIDER strategy, illustrated in Table 1, guided the development of review questions such as: “Does e-cigarette use negatively impact human barrier systems (e.g., respiratory, cardiovascular, gastrointestinal) compared to non-use, and what specific adverse effects have been observed?”.

Following the formulation of the review questions, the authors established an a priori protocol, as described by van den Akker et al.⁵² Additionally, this systematic review has been reported in accordance with the PRISMA guidelines, where applicable.^{53,54} Following these guidelines ensures the transparency and reproducibility of the methodology used in this study.

A comprehensive search was performed in two electronic databases: PubMed, and Scopus.⁵³

The search strategy on PubMed combined the MeSH terms: ("E-Cigarette Vapor"[Mesh] OR "Electronic Nicotine Delivery Systems"[Mesh] OR "Vaping"[Mesh]) AND "Cardiovascular System"[Mesh].

The search on Scopus was carried out using: (TITLE-ABS-KEY (blood AND air AND barrier) OR TITLE-ABS-KEY (blood AND aqueous AND barrier) OR TITLE-ABS-KEY (blood AND brain AND barrier) OR TITLE-ABS-KEY (blood AND retinal AND barrier) OR TITLE-ABS-KEY (blood AND testis AND barrier) OR TITLE-ABS-KEY (glomerular AND filtration AND barrier) OR TITLE-ABS-KEY (glymphatic AND system) AND TITLE-

Table 1
Description of the SPIDER framework used in the systematic review to define the eligibility criteria.

(S) Sample	Studies involving individuals (adolescents, adults, current smokers, and non-smokers), animal models, and in vitro studies involving human or animal cells or tissues exposed to e-cigarette vapor.
(PI) Phenomenon of Interest	The impact of e-cigarette use on barrier systems, including respiratory, cardiovascular, and gastrointestinal barriers.
(D) Design	Various study designs, including qualitative, quantitative, and mixed methods studies, focusing on the effects of e-cigarettes.
(E) Evaluation	Outcomes such as inflammation, permeability changes, oxidative stress, and other adverse effects on barrier systems.
(R) Research type	Inclusion of experimental studies, observational studies, reviews, and in vitro studies.

ABS-KEY (e AND cigarettes) OR TITLE-ABS-KEY (e AND cigarette) OR TITLE-ABS-KEY (electronic AND nicotine) OR TITLE-ABS-KEY (vaping) OR TITLE-ABS-KEY (nicotine AND vaping AND products)).

Study selection process and eligibility criteria

Following the initial identification of search results, peer-reviewed citations were extracted from major databases. The Rayyan software⁵⁵ was utilized to streamline the removal of duplicate records and facilitate the review and management of included articles. Three independent reviewers (R.F.; S.T.; and C.L.) participated in the selection process.

The screening procedure was conducted in two distinct stages. During the initial stage, two independent reviewers (R.F. and S.T.) separately evaluated the titles, abstracts, and keywords of the collected papers to determine their relevance and suitability for inclusion in the systematic review. Articles deemed unrelated to the scope of this systematic review were excluded. Relevant review articles were considered during this stage for potential citation searching, serving as valuable sources for articles not available in the three databases.⁵⁶

In the secondary stage of screening, the same reviewers assessed the full-text manuscripts based on the predefined inclusion and exclusion criteria to identify eligible studies. Any discrepancies between the two reviewers were resolved by the third reviewer (C.L.), who oversaw the review process and provided consultation throughout both stages.

Included studies had to meet the following criteria: (1) original research articles, (2) studies investigating the effects of e-cigarette use on barrier systems (e.g., respiratory, cardiovascular, gastrointestinal), and (3) studies published in English. Exclusion criteria were (1) reviews, case reports, conference papers, and editorials, as well as (2) studies that lacked sufficient data for meaningful analysis. The study selection

adhered to the PRISMA guidelines.^{53,54}

Data were extracted by two independent reviewers (R.F. and S.T.) using a predefined extraction form. Disagreements between the reviewers were resolved through discussion or consultation with a third reviewer (C.L.).

Results

Literature search

A comprehensive search across multiple databases initially identified 149 records. The PRISMA flowchart delineates the selection process of pertinent publications at each stage of the systematic review (Fig. 1). Following the removal of 12 duplicate records, 137 records proceeded to the screening phase. During this phase, records that did not meet the predefined inclusion criteria were excluded (n=111), resulting in 26 reports being assessed for eligibility. Ultimately, all 22 studies were incorporated into the qualitative synthesis. This meticulous selection process ensures the inclusion of high-quality and relevant studies, thereby providing a robust foundation for the review's conclusions.

Characteristics of the included studies

This systematic review encompasses 22 studies that investigate the impact of e-cigarettes on various human barrier systems, such as the blood-brain barrier (BBB), alveolar-capillary barrier, vascular system, and intestinal barrier, analyzing both local and systemic effects. These studies provide diverse insights into the mechanisms by which e-cigarette exposure leads to barrier dysfunction, including inflammation, oxidative stress, and permeability alterations (Table 2).

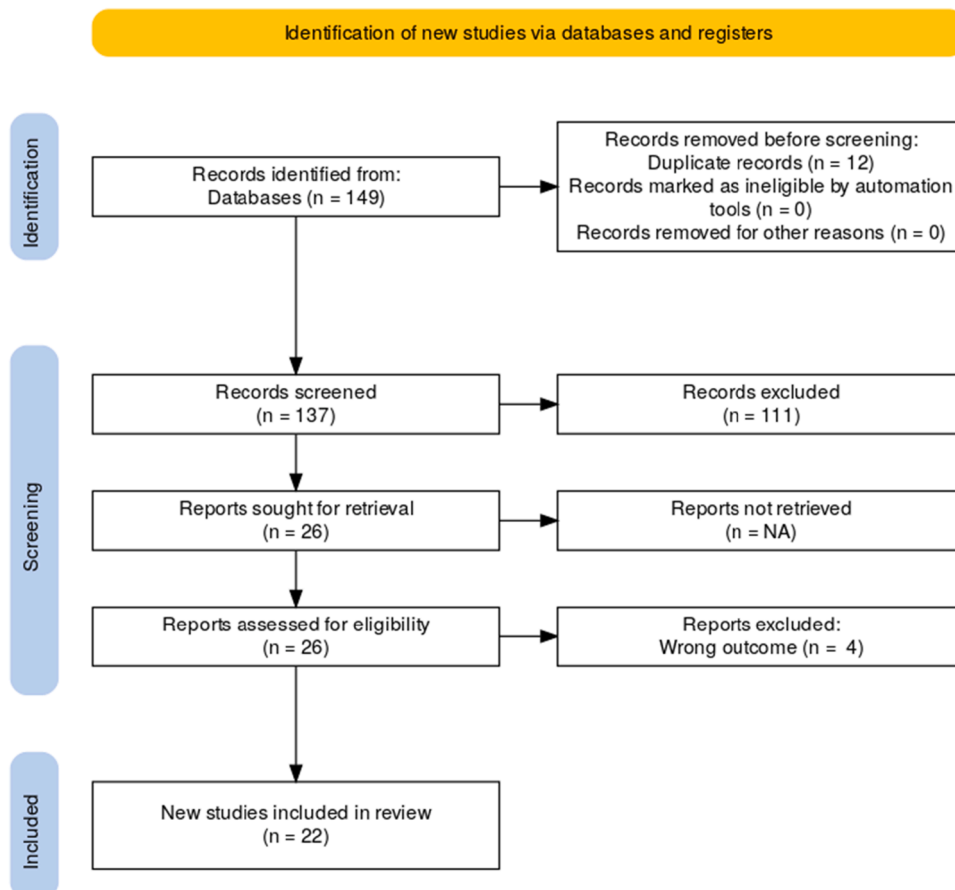


Fig. 1. PRISMA flow diagram of study selection.

Table 2
Studies evaluation.

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Aboaziza et al. 2023 ⁵⁸	Pregnant Sprague–Dawley rats and their offspring.	Long-term effects of maternal e-cigarette use during pregnancy on the arterial function of offspring.	Controlled exposure of pregnant rats to e-cigarette vapor (both nicotine-free and nicotine-containing) and subsequent evaluation of arterial function in their offspring.	/	/	Evaluated through the involvement of reactive oxygen species in impaired aortic relaxation.	Arterial stiffness, endothelial dysfunction, changes in elastin and collagen content in the aorta, and impaired aortic reactivity.	Experimental study on animal model.	- Maternal vaping (with or without nicotine) leads to increased arterial stiffness and impaired aortic relaxation in offspring. - These effects are associated with oxidative stress and reduced nitric oxide bioavailability. - Structural changes in the aorta, such as reduced elastin and increased collagen content, were observed.
Ahmad et al. 2019 ⁵⁷	Male Sprague–Dawley rats	Acute pulmonary effects of aerosolized nicotine, a principal component of e-cigarettes.	Controlled exposure of rats to aerosolized nicotine and subsequent evaluation of pulmonary injury and inflammation.	Increased levels of inflammatory markers such as IL-1A, CXCL1, and myeloperoxidase (MPO).	Increased lung wet-to-dry weight ratio, elevated protein and IgM content in bronchoalveolar lavage fluid (BALF), and decreased E-cadherin expression indicating disruption of the alveolar-capillary barrier.	/	Pulmonary edema, increased neutrophil infiltration, and increased lactate levels in arterial blood.	In vivo assessments of pulmonary function and structure in rats	- Acute inhalation of nicotine aerosol causes significant pulmonary injury, including increased pulmonary edema, lung injury, and inflammation. - Nicotine exposure leads to increased levels of inflammatory markers and disruption of the alveolar-capillary barrier. - The study highlights the potential adverse pulmonary and systemic effects of nicotine, a key component of e-cigarettes.
Archie et al. 2023 ⁶⁴	Pregnant CD1 mice and their offspring	Effects of maternal e-cigarette use on postnatal BBB integrity and behavioral outcomes in offspring.	Controlled exposure of pregnant mice to e-cigarette vapor containing 2.4% nicotine and subsequent evaluation of BBB integrity and behavioral outcomes in their offspring	/	Expression of tight junction proteins (ZO-1, claudin-5, occludin), astrocyte marker (GFAP), pericyte marker (PDGFR β), basement membrane proteins (laminin α 1 and laminin α 4), water channel protein	/	Impaired motor, learning, and memory functions in offspring, as well as reduced body weight and brain-to-body weight ratio	In vivo assessments of BBB integrity and behavioral outcomes in mice	Maternal e-cigarette exposure leads to reduced expression of tight junction proteins (ZO-1, claudin-5, occludin) and astrocyte marker (GFAP) in offspring, indicating disrupted BBB integrity.

(continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
					(AQP4), and glucose transporter (GLUT-1).				• Prenatal e-cigarette exposure results in impaired motor, learning, and memory functions in both male and female offspring. • The study highlights the potential long-term neurovascular changes and behavioral deficits associated with prenatal e-cigarette exposure.
Burrage et al. 2021 ⁵⁹	Pregnant Sprague-Dawley rats and their offspring	Long-term effects of maternal e-cigarette use during pregnancy on cerebrovascular function in offspring.	Controlled exposure of pregnant rats to e-cigarette vapor (both nicotine-free and nicotine-containing) and subsequent evaluation of cerebrovascular function in their offspring.	/	/	Endothelial-dependent dilation (EDD) responses.	Impaired endothelial-dependent dilation of the middle cerebral artery (MCA), increased extracellular vehicles (EVs) in plasma, and no significant differences in body mass or organ weights	In vivo assessments of cerebrovascular function and structure in offspring	• Maternal vaping (with or without nicotine) leads to significant impairment in endothelial-dependent dilation of the MCA in offspring, indicating cerebrovascular dysfunction. • The impairment persists into adolescent and adult life stages. • The study suggests that components of the e-cigarette base solution (other than nicotine) are responsible for the observed cerebrovascular dysfunction.
Chen et al. 2002 ⁶⁵	Human brain microvascular endothelial cells (HBMEC)	How nicotine affects the invasion of E. coli in human brain microvascular endothelial cells, which are a model for the BBB.	Treating HBMEC with various concentrations of nicotine and examining the effects on E. coli invasion, cytoskeletal rearrangement, and signaling pathways.	/	Effects on transendothelial electrical resistance (TEER) and E. coli penetration	/	Enhanced E. coli invasion, actin cytoskeleton rearrangement, and activation of signaling pathways involving PI3 K and ROCK.	In vitro assessments of BBB integrity and cellular responses to nicotine and E. coli.	• Nicotine significantly enhances E. coli invasion of HBMEC in a dose- and time-dependent manner. • The enhancement is associated with actin cytoskeleton rearrangement and morphological changes in HBMEC. • Nicotine-induced enhancement of E. coli invasion is mediated by nicotinic acetylcholine receptors (nAChRs) (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Crotty Alexander et al. 2018 ³¹	- Animal Models: C57BL/6 and CD-1 mice. - In Vitro Studies: Primary human bronchial epithelial cells (NHBEs).	Effects of chronic inhalation of e-cigarette vapor containing nicotine on airway barrier function, systemic inflammation, and multiorgan fibrosis.	Chronic exposure of mice to e-cigarette vapor and in vitro exposure of human bronchial epithelial cells to e-cigarette vapor, followed by assessments of airway permeability, systemic inflammation, and organ fibrosis.	Circulating proinflammatory cytokines and bronchoalveolar lavage fluid analysis.	Transepithelial electrical resistance (TEER) and FITC-dextran permeability assays in NHBEs	/	Reduced renal filtration, increased fibrosis in kidneys, heart, and liver, altered cardiovascular function, and changes in gene expression related to fibrosis.	In vivo assessments of organ function and fibrosis in mice, and in vitro assessments of airway epithelial barrier function.	and involves the PI3 K signaling pathway. - Inhibition of PI3 K blocks the nicotine-induced enhancement of <i>E. coli</i> invasion, while inhibition of ROCK enhances it. - Nicotine and <i>E. coli</i> synergistically induce actin condensation in HBMEC, which is crucial for bacterial entry. - Chronic inhalation of e-cigarette vapor leads to increased circulating proinflammatory and profibrotic proteins, indicating systemic inflammation. - E-cigarette vapor exposure reduces airway barrier function, leading to increased permeability and higher secretion of proinflammatory cytokines in response to bacterial infection. - Chronic e-cigarette vapor inhalation induces renal dysfunction and fibrosis, as well as cardiac fibrosis and altered cardiovascular function. - The study suggests that e-cigarette vapor may have proinflammatory and profibrotic effects on multiple organs, potentially leading to long-term health consequences. - Gestational exposure to e-cigarette aerosol and HFD triggers significant CM (continued on next page)
Hasan et al. 2021 ⁶⁶	Time-mated pregnant mice and their male offspring	Effects of gestational exposure to e-cigarette aerosol containing nicotine	Exposing pregnant mice to e-cigarette aerosol with 2.4% nicotine and a high-fat	/	/	Expression of 4-hydroxynonenal (4-HNE) as a	Increased CM apoptosis, inactivation of AMP-activated	In vivo assessments of cardiac function and structure in neonatal mice.	

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
		and a high-fat diet (HFD) on the hearts of neonatal mice	diet from embryonic day 4 (E4) to E20, followed by assessments of cardiomyocyte (CM) apoptosis, oxidative stress, and ultrastructural abnormalities in the hearts of their offspring at postnatal days 1 (PND1), 3 (PND3), and 14 (PND14).			biomarker of oxidative stress.	protein kinase (AMPK), perturbation of BAX/BCL-2 ratio, activation of caspase 3, and ultrastructural abnormalities in the heart.		apoptosis in neonatal hearts, particularly at PND3 and PND14. • This exposure leads to inactivation of AMPK, increased oxidative stress, and perturbation of the BAX/BCL-2 ratio, resulting in the activation of caspase 3. • Ultrastructural abnormalities in the hearts of exposed pups include shrunken nuclei with chromatin condensation, myofibrillar thinning, and enlarged mitochondria with signs of cristolysis, indicative of cardiomyopathy and cardiac dysfunction.
Heldt et al. 2020 ⁴³	Male C57BL/6 mice.	Effects of long-term e-cigarette exposure on BBB integrity, neuroinflammation, and cognitive function	Exposing mice to e-cigarette vapor with and without nicotine, as well as traditional cigarette smoke, followed by assessments of BBB function, gene expression, protein localization, leukocyte-endothelial interactions, and cognitive performance.	Evaluated through leukocyte-endothelial cell interactions and microglial activation.	Assessed using sodium fluorescein (NaF) and tetramethylrhodamine (TMR)-dextran tracers.	/	Reduced expression of tight junction proteins (Occludin), glucose transporter (Glut1), increased BBB permeability, and impaired cognitive function.	In vivo assessments of BBB integrity, neuroinflammation, and cognitive function in mice	• E-cigarette exposure, particularly nicotine-free e-cigarettes, leads to significant down-regulation of genes critical for BBB function and reduced protein expression of Occludin and Glut1. • Increased BBB permeability is observed following exposure to nicotine-free e-cigarettes, while nicotine-containing e-cigarettes and traditional cigarettes also disrupt BBB integrity but to a lesser extent. • Pro-inflammatory changes are noted, with increased leukocyte-endothelial interactions and microglial activation, particularly in the striatum. (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Kadry et al. 2021 ⁷⁵	Induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells (BMECs).	Effects of cigarette smoke (TSe) and e-cigarette vapor extract (ECe) on the integrity of BBB and evaluates the protective effects of metformin.	Exposing iPSC-derived BMECs to TSe and ECe, with and without metformin pre-treatment, followed by assessments of BBB permeability, tight junction protein expression, and distribution.	/	Assessed using sucrose and mannitol as paracellular markers and transendothelial electrical resistance (TEER) measurements.	/	Changes in the expression and distribution of tight junction proteins (ZO-1, Occludin, Claudin-5).	In vitro assessments of BBB integrity and the protective effects of metformin.	• Cognitive impairments are observed in mice exposed to nicotine-free e-cigarettes, as evidenced by deficits in the novel object recognition test. • Both TSe and ECe negatively impact BBB integrity, increasing permeability to sucrose and mannitol and reducing TEER values. • The overall expression levels of ZO-1 and Occludin were not significantly down-regulated, but their distribution was altered, showing discontinuation patterns. • Claudin-5 expression was significantly decreased by both TSe and ECe. • Metformin pre-treatment effectively protected BBB integrity against TSe and ECe exposure, maintaining TEER values and preventing the downregulation and dysregulation of tight junction proteins.
Kaisar et al. 2017 ⁶⁸	• Animal Models: C57BL/6 J male mice. • In Vitro Model: Mouse primary brain microvascular endothelial cells (mBMEC).	Effects of tobacco smoke (TS) and e-cigarette vapor (e-Cig) on BBB integrity, oxidative stress, inflammation, and stroke injury, and evaluates the protective effects of metformin (MF).	Exposing mice to TS and e-Cig vapor, with and without metformin treatment, followed by assessments of BBB integrity, oxidative stress, inflammation, and stroke injury. In vitro studies were conducted using mBMECs to evaluate cellular responses to TS and e-Cig extracts.	Evaluated through the expression of pro-inflammatory markers (PECAM-1, ICAM-1, VCAM-1) and TNF- α levels.	Assessed using transendothelial electrical resistance (TEER) measurements and permeability assays with fluorescent dextrans.	Evaluated through the activation of Nrf2 and its downstream signaling molecule NQO-1.	Changes in the expression and distribution of tight junction proteins (ZO-1), infarct size, and neurological deficits following stroke.	In vivo and in vitro assessments of cerebrovascular responses to TS and e-Cig exposure and the protective effects of metformin	• Both TS and e-Cig exposure promote oxidative stress, as evidenced by increased cellular oxidative stress and activation of Nrf2 and NQO-1. • TS and e-Cig exposure negatively impact BBB integrity, reducing TEER values and increasing permeability to fluorescent dextrans.

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Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
									• The expression of tight junction protein ZO-1 is down-regulated by both TS and e-Cig exposure, indicating BBB impairment. • Pro-inflammatory markers (PECAM-1, ICAM-1, VCAM-1) are upregulated in response to TS and e-Cig exposure, both in vitro and in vivo. • Chronic exposure to TS and e-Cig increases the risk of stroke and worsens post-ischemic brain injury, as demonstrated by increased infarct size and neurological deficits. • Metformin treatment provides partial protection against the harmful effects of TS and e-Cig exposure, reducing oxidative stress, inflammation, BBB impairment, and stroke injury.
Kaisar et al. 2018 ⁶⁹	• Animal Models: C57BL/6 J male and female mice. • In Vitro Model: Mouse primary brain microvascular endothelial cells (mBMEC) and bEnd.3 cell line.	Effects of tobacco smoke (TS) and e-cigarette vapor (e-Cig) on mitochondrial function, iron transporters, and detoxifying enzymes at the brain vascular endothelium, with a focus on gender-specific responses to chronic TS exposure.	exposing mice to TS and e-Cig vapor, followed by assessments of mitochondrial membrane potential, expression of iron transporters (Slc40a1, Abcb6), and phase-II detoxifying enzymes (GSTP1, GSTA4). In vitro studies were conducted using mBMECs and bEnd.3 cells to evaluate cellular responses to TS and e-Cig extracts.	/	/	Evaluated through mitochondrial membrane potential and expression of iron transporters.	Changes in the expression of detoxifying enzymes (GSTP1, GSTA4) and mitochondrial depolarization.	In vivo and in vitro assessments of cerebrovascular responses to TS and e-Cig exposure, with a focus on gender-specific differences.	• Both TS and e-Cig exposure promote mitochondrial depolarization in brain vascular endothelial cells, indicating mitochondrial dysfunction. • TS and e-Cig exposure upregulate the expression of the transmembrane iron exporter Slc40a1 and the mitochondrial ATP-binding cassette porphyrin importer Abcb6, which are crucial for maintaining cellular iron and redox homeostasis.

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Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Lockman et al. 2005 ⁶⁰	Male Fischer-344 rats	How chronic nicotine exposure affects the BBB permeability and the brain uptake of MLA, a selective antagonist for the nicotinic acetylcholine receptor $\alpha 7$ subtype.	Involves exposing rats to chronic nicotine (4.5 mg/kg/day for 28 days) using osmotic minipumps, followed by assessments of BBB permeability and brain uptake of MLA using in situ rat brain perfusions.	/	Assessed using the brain uptake rates of MLA and the passive permeation of thiourea.	/	Changes in the brain uptake of MLA and the passive diffusion of compounds with very low extraction rates.	In vivo assessments of BBB permeability and brain uptake of MLA in rats exposed to chronic nicotine	• Chronic TS exposure results in gender-specific differential responses in the expression of iron transporters and phase-II detoxifying enzymes. In female mice, TS exposure significantly down-regulates Slc40a1 and GSTP1, while upregulating Abcb6 and GSTA4. In male mice, these changes are less pronounced or absent. • The study suggests that gender is a risk factor that needs to be considered when assessing the potential impact of chronic smoking and e-Cig vaping on cerebrovascular health. • Chronic nicotine exposure significantly diminishes the brain uptake of MLA by 60%. • This reduction in MLA brain uptake is not related to nicotine-induced MLA brain efflux or BBB transport alterations. • Chronic nicotine exposure also diminishes the passive permeation of thiourea by 24%, indicating decreased BBB permeability. • The study suggests that chronic nicotine exposure may diminish the blood-brain passive diffusion of compounds with very low extraction rates, implying that the pharmacokinetics

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Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Mandour et al. 2021 ⁷⁴	30 adult male albino rats.	Histopathological changes in the submandibular gland of rats exposed to electronic cigarette (E-cig) vapor and the potential amelioration after cessation of exposure.	Exposing rats to E-cig vapor (1 ml/day for 1 hour, 5 days a week for four weeks), followed by assessments of histological and biochemical changes in the submandibular gland. A withdrawal group was also included to evaluate the potential recovery after cessation of exposure.	Assessed using immunohistochemical staining for Tumor Necrosis Factor- α (TNF- α).	/	Evaluated through serum malondialdehyde (MDA) levels and Glutathione peroxidase-1 (GPx1) enzyme activity.	Histopathological changes in the submandibular gland, including vacuolated cytoplasm, thickened connective tissue septa, and dilated excretory ducts.	In vivo assessments of histopathological and biochemical changes in the submandibular gland of rats exposed to E-cig vapor.	of neuro-pharmaceutical agents that are permeability-limited may need to be re-evaluated in individuals exposed to nicotine. - E-cig vapor exposure caused significant histopathological changes in the submandibular gland, including vacuolated cytoplasm, thickened connective tissue septa, and dilated excretory ducts. - There was an increase in TNF-α immunoreactivity and its area percentage, indicating inflammation. - E-cig vapor exposure resulted in oxidative damage, as evidenced by increased MDA levels and decreased GPx1 enzyme activity. - Cessation of E-cig vapor exposure led to some amelioration of the histological and biochemical changes, although not complete recovery.
Marinucci et al. 2020 ⁷⁰	Purified pig pre-pubertal Sertoli cells (SC)	Effects of nicotine on the viability and function of purified pig pre-pubertal Sertoli cells, focusing on extracellular matrix (ECM) and tight junction protein gene expression, and BTB permeability.	Exposing Sertoli cells to various concentrations of nicotine (0.1, 1, and 10 μ M) for 48 hours, followed by assessments of cell viability, function, and gene expression related to ECM and tight junction proteins.	/	Assessed through gene expression levels of occludin, integrin β 1, integrin α 6, laminin, and collagen IV.	/	Changes in the secretion of anti-Mullerian hormone (AMH) and inhibin B, and the expression of follicle-stimulating hormone receptor (FSH-r) and aromatase.	In vitro assessments of Sertoli cell viability, function, and gene expression in response to nicotine exposure.	- Nicotine exposure significantly reduced the mRNA expression and protein levels of AMH and inhibin B, indicating impaired Sertoli cell function. - Nicotine impaired FSH-r sensitivity via the downregulation of FSH-r and aromatase gene expression. - Nicotine exposure altered ECM and tight junction protein gene expression, (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Mekala et al. 2022 ⁶⁷	Human brain microvascular endothelial cells (hBMVECs)	How chronic exposure to alcohol (ETH), acetaldehyde (ALD), and e-Cig affects mitochondrial function and barrier integrity in hBMVECs, and the protective role of P2 × 7 receptor (P2 × 7r) antagonist	Treating hBMVECs with ETH (100 mM), ALD (100 µM), or e-Cig (35 ng/mL of 1.8% or 0% nicotine) conditioned medium, followed by assessments of mitochondrial oxidative phosphorylation (OXPHOS), mitochondrial membrane potential, intracellular calcium levels, extracellular ATP release, and barrier function. The protective effects of P2 × 7r antagonist A804598 (10 µM) were also evaluated.	Assessed through gene expression of P2 × 7r and TRPV1 channels.	Evaluated using trans-endothelial electrical resistance (TEER) assay.	Assessed through mitochondrial OXPHOS levels, mitochondrial membrane potential, and intracellular calcium levels.	Changes in mitochondrial OXPHOS protein expression, extracellular ATP release, and endoplasmic reticulum (ER) stress markers.	In vitro assessments of mitochondrial function, oxidative stress, and barrier integrity in hBMVECs exposed to ETH, ALD, and e-Cig, with and without P2 × 7r antagonist treatment.	compromising cross-talk between the interstitial and tubular compartments and enhancing BTB permeability. - The study suggests that nicotine can significantly alter the expression of genes related to ECM components (e.g., laminin, integrin, and occludin), thus compromising BTB integrity via downregulation of the mitogen-activated protein kinase (MAPK) pathway. - Nicotine exposure increased early and late apoptotic cells and necrotic cells, indicating cytotoxic effects. - ETH, ALD, and 1.8% e-Cig exposure significantly reduced mitochondrial OXPHOS levels and impaired mitochondrial function in hBMVECs. - These exposures led to increased intracellular calcium levels, ER stress, and upregulation of pro-apoptotic factors. - P2 × 7r antagonist A804598 effectively restored mitochondrial function, reduced intracellular calcium levels, and prevented ER stress and pro-apoptotic signaling. - Blocking P2 × 7r also prevented the extracellular release of ATP and protected (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Mekala et al. 2024 ⁷²	Primary human pulmonary alveolar epithelial cells (hPAEpiC) and human brain microvascular endothelial cells (hBMVECs).	How chronic exposure to alcohol (ETH), acetaldehyde (ALD), and e-Cig affects mitochondrial function, EV release, and paracrine signaling in hPAEpiC, and the subsequent impact on hBMVECs	Treating hPAEpiC with ETH (100 mM), ALD (100 µM), or e-Cig (1.75 µg/mL of 1.8% or 0% nicotine) conditioned medium, followed by assessments of mitochondrial efficiency, gene expression, EV release, and EV cargo. The effects of these EVs on hBMVECs were also evaluated. The protective effects of P2 × 7 receptor (P2 × 7r) antagonist A804598 (10 µM) were assessed.	Assessed through gene expression of P2 × 7r and TRPV1 channels, and ER stress markers.	Evaluated through EV release and cargo analysis.	Assessed through mitochondrial oxidative phosphorylation (OXPHOS) levels, mitochondrial membrane potential, and intracellular calcium levels.	Changes in EV size and number, extracellular ATP (eATP) release, and mitochondrial DNA (mtDNA) content in EVs.	In vitro assessments of mitochondrial function, oxidative stress, EV release, and paracrine signaling in hPAEpiC and hBMVECs exposed to ETH, ALD, and e-Cig, with and without P2 × 7r antagonist treatment	barrier integrity, as evidenced by improved TEER values. • The study suggests that P2 × 7r plays a crucial role in mediating mitochondrial dysfunction and barrier disruption caused by ETH, ALD, and e-Cig exposure. • ETH, ALD, and 1.8% e-Cig exposure significantly reduced mitochondrial OXPHOS levels and impaired mitochondrial function in hPAEpiC. • These exposures led to increased expression of P2 × 7r and TRPV1 channels, elevated intracellular calcium levels, and upregulated ER stress markers. • ETH, ALD, and 1.8% e-Cig exposure increased the release of EVs with larger particle sizes, carrying higher amounts of eATP and mtDNA. • EVs from insult-exposed hPAEpiC triggered paracrine signaling in hBMVECs, increasing intracellular calcium levels. • P2 × 7r antagonist A804598 effectively restored mitochondrial function, reduced ER stress, and diminished EV release and cargo, thus protecting BBB function. (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Orzabal et al. 2019 ⁷¹	Timed-pregnant Sprague-Dawley rats	How chronic exposure to e-cig aerosols containing nicotine during pregnancy affects fetal and postnatal growth, as well as maternal and fetal vascular hemodynamics.	Exposing pregnant rats to e-cig aerosols with and without nicotine using a custom-engineered vaping system. The exposure paradigms include prenatal-only and prenatal + postnatal exposure. Growth measurements and vascular hemodynamics were assessed using Doppler ultrasonography and other techniques.	/	Evaluated through blood flow measurements in maternal uterine and fetal umbilical arteries.	/	Changes in fetal and pup weight, crown-rump length, and serum nicotine levels.	In vivo assessments of growth and vascular hemodynamics in offspring of rats exposed to e-cig aerosols during gestation, with and without nicotine.	• The study suggests that $P2 \times 7r$ plays a crucial role in mediating mitochondrial dysfunction, EV release, and paracrine signaling caused by ETH, ALD, and e-Cig exposure. • Chronic exposure to e-cig aerosols containing nicotine during early development significantly decreased fetal weight and crown-rump length. • Prenatal and postnatal exposure to e-cig aerosols with nicotine resulted in decreased pup weight and crown-rump length at postnatal day (PND) 10. • Blood flow in the maternal uterine and fetal umbilical arteries was significantly decreased in the Juice+Nicotine group, indicating potential growth restriction. • The study suggests that vaping e-cigs containing nicotine during pregnancy can lead to harmful developmental effects, including intrauterine growth restriction (IUGR) and reduced nutrient delivery to the fetus.
Shah et al. 2015 ⁶³	CD-1 male mice	How nicotine pre-exposure affects GLUT1 function and expression at the BBB in a focal brain ischemia model.	Administering nicotine subcutaneously via osmotic pump at a dose of 4.5 mg/kg/day for 1, 7, or 14 days to	/	Evaluated through glucose transport rates and GLUT1 expression at the BBB.	/	Changes in glucose transport rates and GLUT1 expression in response to nicotine pre-	In vivo assessments of glucose transport and GLUT1 expression at the BBB in mice pre-exposed to nicotine	• Nicotine pre-exposure for 1, 7, and 14 days significantly reduced the glucose influx rate (K_{in}) across the BBB, with a 49% reduction

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Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
			reflect plasma levels seen in smokers. Ischemic-reperfusion (IR) injury was induced by 1-hour transient middle cerebral artery occlusion (tMCAO) followed by 24-hour reperfusion. Glucose transport was estimated using an in situ brain perfusion technique with radiolabeled glucose, and brain vascular GLUT1 expression was detected with immunofluorescence.				exposure and ischemic-reperfusion injury.	and subjected to ischemic-reperfusion injury.	in 14-day nicotine-infused animals. • In saline-infused tMCAO animals, glucose transport across the BBB increased by 41% compared to saline-infused sham animals. • Nicotine pre-exposure for 14 days significantly attenuated the increased glucose transport in tMCAO animals by 80% and 38% compared to saline-infused tMCAO and sham animals, respectively. • Immunofluorescence studies confirmed that GLUT1 protein expression was induced in saline-infused tMCAO animals and significantly reduced with 14-day nicotine pre-exposure in tMCAO animals. • The study suggests that nicotine exposure prior to stroke could create an enhanced glucose-deprived state at the neurovascular unit (NVU), potentially increasing vulnerability to stroke injury.
Sifat et al. 2018 ⁶¹	• In Vitro Model: Mouse primary cortical neurons. • In Vivo Model: C57BL/6 J male mice.	How nicotine and e-Cig exposure affect neuronal glucose utilization and glucose transporter expression in ischemic conditions	Treating primary cortical neurons with nicotine and cotinine, followed by oxygen-glucose deprivation/reoxygenation (OGD/R) to mimic ischemic stroke. Additionally, mice were exposed to e-Cig vapor, and brain slices were subjected to OGD/R. Glucose utilization was measured using [3H]	/		Evaluated through glucose transporter expression and glucose uptake.	/	Changes in glucose uptake, glycolytic capacity, and oxygen consumption rate (OCR) in response to nicotine and e-Cig exposure.	In vitro and in vivo/ex vivo assessments of glucose utilization and glucose transporter expression in neurons and brain slices exposed to nicotine and e-Cig vapor under ischemic conditions. • Nicotine and cotinine exposure significantly decreased neuronal glucose uptake in ischemic conditions, with both short-term and long-term treatment. • Nicotine/cotinine exposure decreased neuronal GLUT1 expression and upregulated $\alpha 7$ nicotinic

(continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
			deoxy-D-glucose uptake, and glucose transporter expression was assessed using immunofluorescence and western blotting.						acetylcholine receptor (nAChR) expression in ischemic conditions. • Nicotine/cotinine treatment reduced neuronal glycolytic capacity and mitochondrial respiration in ischemic conditions. • Mice exposed to e-Cig vapor for 7 days showed decreased brain glucose uptake and reduced expression of GLUT1 and GLUT3 in brain slices under normoxic and ischemic conditions. • The study suggests that nicotine and e-Cig exposure induce a state of glucose deprivation at the neurovascular unit, potentially leading to enhanced ischemic brain injury and increased stroke risk.
Sifat et al. 2022 ⁶²	Male C57BL/6 mice	How short-term exposure to JUUL e-Cigs affects brain injury, BBB tight junction proteins, and inflammatory and oxidative stress markers in ischemic stroke, compared to tobacco smoke (TS) exposure.	Exposing mice to JUUL e-Cig vapor or TS for 14 days. Ischemic stroke was induced using transient middle cerebral artery occlusion (tMCAO) followed by reperfusion. Plasma levels of nicotine, cotinine, IL-6, and thrombomodulin were measured. Western blotting and immunofluorescence were used to study BBB tight junction proteins and key inflammatory and oxidative stress markers.	Assessed through plasma IL-6 levels and brain ICAM-1 expression.	Evaluated through the expression of BBB tight junction proteins (claudin-5, occludin, ZO-1).	Assessed through brain Nrf2 expression.	Changes in brain injury, swelling ratio, neurological score, and plasma thrombomodulin levels.	In vivo assessments of brain injury, BBB integrity, and inflammatory and oxidative stress markers in mice exposed to JUUL e-Cigs or TS, followed by ischemic stroke.	• Plasma nicotine and cotinine levels were significantly higher in TS-exposed mice compared to JUUL-exposed mice. • Both JUUL and TS exposure caused weight reduction in mice, with TS having a more pronounced effect. • JUUL and TS exposure worsened brain injury 24 hours after MCAO, with TS exposure also increasing brain swelling and worsening neurological function. • Plasma IL-6 levels increased and thrombomodulin levels (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
									decreased after MCAO, with TS exposure having a more significant effect on IL-6 levels. - JUUL and TS exposure reduced the expression of BBB tight junction proteins (ZO-1, claudin-5, occludin) after MCAO, with JUUL having a more significant effect on occludin expression. - JUUL and TS exposure decreased Nrf2 expression in the brain after MCAO, with TS exposure also increasing ICAM-1 expression in the ischemic brain hemisphere. - The study suggests that JUUL exposure can negatively impact the cerebrovascular system, although to a lesser extent than TS exposure.
Sivandzade et al. 2019 ⁷⁶	Mouse primary brain microvascular endothelial cells (mBMECs).	How RSG, an anti-diabetic drug, can protect against BBB impairment caused by TS and e-Cig exposure	Treating mBMECs with RSG and exposing them to TS and e-Cig extracts. The effects on BBB integrity, oxidative stress, inflammation, and tight junction protein expression were assessed using various techniques, including Western blotting, immunofluorescence, TEER measurement, and permeability assays.	Assessed through the expression of the inflammatory adhesion molecule PECAM-1.	Evaluated through the expression of tight junction proteins (ZO-1) and BBB integrity using TEER and permeability assays.	Assessed through intracellular ROS generation and the expression of Nrf2 and its downstream effector NQO-1.	Changes in PPAR γ expression and overall BBB integrity.	In vitro assessments of BBB integrity, oxidative stress, inflammation, and tight junction protein expression in mBMECs exposed to TS and e-Cig extracts, with and without RSG treatment	- RSG significantly decreased intracellular ROS generation and reduced the pro-inflammatory activity of TS and e-Cig extracts. - RSG upregulated PPARγ expression and enhanced Nrf2 activity, leading to increased expression of the downstream effector NQO-1. - TS and e-Cig exposure downregulated the expression of the tight junction protein ZO-1, which was countered by RSG treatment. (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation				(R) Research type	Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect		
Zhang et al. 2023 ⁷³	Eight-week-old Balb/c male mice.	How nicotine exposure affects ferroptosis, a type of regulated cell death, and the integrity of the BTB in male mice	Administering nicotine to mice via intraperitoneal injection to mimic the blood plasma levels of nicotine in heavy smokers. The effects on BTB integrity, ferroptosis, and sperm parameters were assessed using various techniques, including transmission electron microscopy (TEM), immunofluorescence staining, and biochemical assays.	/	Evaluated through the expression of BTB-related proteins (ZO-1, N-Cad, Occludin, CX-43) and TEM analysis.	Assessed through the levels of GSH, MDA, and ROS.	Changes in sperm parameters, iron content, and the expression of ferroptosis-related proteins (GPX4, ferritin).	In vivo assessments of BTB integrity, ferroptosis, and oxidative stress in mice exposed to nicotine.	• RSG treatment improved BBB integrity, as demonstrated by increased TEER values and reduced permeability to labeled dextrans. • The study suggests that RSG could be a promising therapeutic agent to prevent TS and e-Cig-induced cerebrovascular dysfunction by enhancing Nrf2 activity and reducing oxidative stress and inflammation. • Nicotine exposure disrupted the circadian rhythm of BTB-related factors and induced ferroptosis in testicular cells. • Nicotine decreased the expression of BTB-related proteins (ZO-1, N-Cad, Occludin, CX-43) and impaired sperm parameters (concentration, vitality, morphology). • Nicotine exposure increased oxidative stress, as evidenced by decreased GSH levels and increased MDA levels. • Nicotine induced iron accumulation and decreased the expression of ferritin and GPX4, leading to ferroptosis. • Inhibition of ferroptosis with Ferrostatin-1 (Fer-1) alleviated nicotine-induced BTB injury and impaired sperm parameters. (continued on next page)

Table 2 (continued)

Reference	(S) Sample	(PI) Phenomenon of Interest.	(D) Design	(E) Evaluation		(R) Research type		Outcome
				Inflammation	Permeability changes	Oxidative stress	Other adverse effect	
								- Nicotine decreased the transcription of Nrf2 through Bmal1, leading to the inactivation of the Nrf2 pathway and its downstream antioxidant genes, resulting in redox imbalance and ROS accumulation. - The study suggests that targeting the Bmal1-Nrf2 axis may help prevent nicotine-induced male reproductive injury.

Pulmonary barrier dysfunction and systemic inflammation

Several studies focus on the alveolar-capillary barrier, highlighting how e-cigarette aerosols compromise lung integrity. Ahmad et al.⁵⁷ demonstrated significant pulmonary inflammation and barrier disruption in male Sprague-Dawley rats following exposure to aerosolized nicotine. Increased levels of inflammatory cytokines such as IL-1A and CXCL1, alongside increased lung permeability, were indicative of lung injury. Furthermore, the study noted oxidative stress as a key mediator in this barrier disruption.

In line with this, Crotty Alexander et al.³¹ explored the systemic impact of chronic e-cigarette exposure, showing elevated inflammatory cytokines in the bronchoalveolar lavage fluid (BALF) and blood, along with significant fibrosis in multiple organs, including the lungs, heart, and liver. This systemic inflammatory response suggests that the impact of e-cigarettes extends beyond localized lung damage, affecting other critical systems.

Vascular system and cardiovascular health

The vascular system was a key focus in many studies, with e-cigarettes shown to affect arterial function, even in the absence of nicotine. Aboaziza et al.⁵⁸ explored the long-term vascular effects of maternal e-cigarette exposure during pregnancy in Sprague-Dawley rats, observing increased arterial stiffness and impaired aortic relaxation in the offspring. These effects were linked to oxidative stress and changes in collagen and elastin content, highlighting the potential cardiovascular risks posed by e-cigarettes.

Additionally, Burrage et al.⁵⁹ demonstrated that maternal exposure to e-cigarettes resulted in long-term cerebrovascular dysfunction in offspring, with impaired endothelial-dependent dilation of the middle cerebral artery (MCA). This dysfunction persisted into adulthood, underscoring the long-lasting impact of e-cigarettes on vascular health, even with nicotine-free aerosols.

Blood-brain barrier disruption and stroke risk

Lockman et al.⁶⁰ explored how chronic nicotine exposure affects BBB permeability and brain uptake of specific compounds. Their findings showed a significant reduction in the brain uptake of the α 7-nicotinic acetylcholine receptor antagonist MLA, highlighting how chronic nicotine exposure diminishes BBB permeability, which could alter the pharmacokinetics of drugs that rely on passive diffusion across the BBB.

Sifat et al.⁶¹ and Sifat et al.⁶² both examined the effects of e-cigarette exposure on stroke outcomes and BBB integrity. Short-term e-cigarette exposure reduced the expression of key tight junction proteins (e.g., claudin-5, ZO-1) and decreased brain glucose utilization, exacerbating ischemic stroke injury. These studies demonstrated that even brief exposure to e-cigarette vapor can significantly worsen stroke outcomes by disrupting glucose metabolism and increasing neuroinflammation.

Shah et al.⁶³ explored how nicotine pre-exposure affects glucose transporter (GLUT1) activity at the BBB in a model of brain ischemia. The study revealed that pre-exposure to nicotine significantly reduced the activity of GLUT1 at the BBB, which compromised glucose transport to the brain, an essential process during ischemic events. Nicotine pre-exposure reduced glucose transport in both sham and ischemic conditions, potentially worsening the energy crisis in brain tissue following stroke and exacerbating ischemic injury.

Blood-Brain Barrier integrity and neuroinflammation

The integrity of the BBB emerged as a major area of concern across multiple studies. Archie et al.⁶⁴ investigated the effects of maternal e-cigarette use on BBB integrity and behavioral outcomes in the offspring of pregnant CD1 mice, finding reduced expression of tight junction proteins such as ZO-1 and occludin, alongside neuroinflammation and behavioral deficits in offspring. Similarly, Heldt et al.⁴³ found that e-cigarette vapor led to increased BBB permeability and microglial activation in male C57BL/6 mice, contributing to cognitive impairments. This study indicated that the inflammatory

response triggered by e-cigarette exposure compromises the protective role of the BBB, with potential consequences for long-term brain function.

Oxidative stress as a central mechanism

Oxidative stress was consistently identified as a critical mechanism underlying the adverse effects of e-cigarettes on barrier systems. Chen et al.⁶⁵ found that nicotine enhanced the invasion of *Escherichia coli* into human brain microvascular endothelial cells (HBMECs) by inducing cytoskeletal rearrangements and activating the PI3K signaling pathway, which resulted in compromised BBB integrity. This highlights how nicotine, a key component of many e-cigarette products, can exacerbate bacterial invasion and barrier disruption.

In the cardiovascular context, Hasan et al.⁶⁶ observed significant cardiomyocyte apoptosis and mitochondrial dysfunction in neonatal mice following gestational exposure to e-cigarette aerosol containing nicotine. The study reported increased oxidative stress, as indicated by elevated levels of 4-hydroxynonenal (4-HNE), a biomarker of oxidative damage, and disruptions in key mitochondrial pathways. This underscores the potential for e-cigarette exposure during pregnancy to induce long-term cardiac damage in offspring, particularly through oxidative stress-mediated mechanisms. Additionally, Mekala et al.⁶⁷ focused on brain microvascular endothelial cells (hBMVECs) and demonstrated that e-cigarette exposure leads to mitochondrial dysfunction, elevated intracellular calcium levels, and increased endoplasmic reticulum (ER) stress. These cellular stress responses are central to the barrier-disrupting effects observed, as impaired mitochondrial function was associated with increased permeability of the BBB. This suggests that mitochondrial health plays a crucial role in maintaining barrier integrity, and e-cigarette components, even in the absence of nicotine, can cause significant cellular dysfunction.

Kaisar et al.⁶⁸ investigated the effects of tobacco smoke (TS) and e-cigarette vapor on the BBB, showing that both TS and e-cigarette vapor promote oxidative stress and BBB impairment. The study demonstrated that exposure to TS and e-cigarettes increased pro-inflammatory markers (e.g., TNF- α , PECAM-1), decreased tight junction proteins like ZO-1, and increased permeability to fluorescent dextrans. Importantly, the study highlighted metformin as a potential therapeutic intervention, partially mitigating these negative effects. Kaisar et al.⁶⁹ further explored gender-specific responses to chronic tobacco smoke exposure, showing that TS and e-cigarette vapor induce mitochondrial depolarization in brain endothelial cells. The study identified sex-specific differences in the regulation of iron transporters and detoxifying enzymes, with more pronounced effects in female mice, which underscores the importance of considering gender in evaluating the cerebrovascular risks of e-cigarette exposure.

Impact on developmental and reproductive health

Several studies explored the effects of prenatal and early-life exposure to e-cigarettes, revealing concerning findings about their impact on developmental health. Burrage et al.⁵⁹ showed that maternal vaping led to long-term cerebrovascular impairments in offspring, including reduced endothelial function in the brain, which persisted into adulthood. This finding suggests that e-cigarettes have the potential to cause enduring damage to the neurovascular system, particularly when exposure occurs during critical periods of development.

Similarly, Hasan et al.⁶⁶ reported that gestational exposure to e-cigarettes, particularly when combined with a high-fat diet, resulted in cardiac apoptosis and oxidative damage in neonatal mice, indicating that the harmful effects of e-cigarettes are compounded by additional lifestyle factors. This highlights the potential for compounded risks during pregnancy, where maternal health and environmental exposures can significantly impact fetal development.

Moreover, Marinucci et al.⁷⁰ focused on the reproductive system by studying the effects of nicotine on Sertoli cells in the testicular environment. The study found that e-cigarette exposure led to increased

apoptosis and altered the expression of tight junction proteins, such as occludin and laminin, compromising the integrity of the blood-testis barrier (BTB). This barrier disruption was associated with impaired reproductive function, suggesting that e-cigarette use may have detrimental effects on male fertility. Orzabal et al.⁷¹ investigated the long-term effects of e-cigarette exposure during pregnancy, finding that exposure to nicotine-containing aerosols led to intrauterine growth restriction and reduced fetal and postnatal growth. The study highlighted decreased blood flow in maternal and fetal arteries, suggesting that maternal vaping could lead to significant vascular dysfunction and growth deficits in offspring.

Mekala et al.^{67,72} expanded on these findings by showing that e-cigarette exposure, particularly when combined with alcohol, damages alveolar-epithelial and brain endothelial cells. This exposure led to mitochondrial dysfunction, increased intracellular calcium levels, and extracellular vesicle (EV) release, which in turn compromised BBB integrity. Blocking P2 $\times$ 7 receptors was shown to protect against this damage, suggesting potential therapeutic pathways.

Zhang et al.⁷³ focused on how nicotine exposure induces ferroptosis, a form of iron-dependent cell death, in BTB integrity. The study revealed that nicotine exposure disrupts BTB integrity by modulating the Bmal1/Nrf2 signaling pathway, which plays a critical role in maintaining cellular redox homeostasis. Disruption of this pathway led to increased oxidative stress, altered tight junction protein expression, and compromised BTB integrity, ultimately affecting male fertility.

Multi-organ effects and fibrosis

Several studies provided evidence of multi-organ effects, extending beyond the immediate barriers studied. Crotty Alexander et al.³¹ demonstrated that chronic e-cigarette exposure caused systemic inflammation and organ fibrosis in the lungs, heart, and kidneys of animal models. This widespread fibrotic response indicates that e-cigarette use can lead to long-term structural changes across various organs, potentially leading to organ dysfunction over time.

Additionally, Hasan et al.⁶⁶ noted mitochondrial abnormalities in the hearts of neonatal mice, suggesting that oxidative stress induced by e-cigarettes plays a pivotal role in the development of fibrosis in vital organs. This finding underscores the broad systemic risks associated with chronic e-cigarette exposure, which affects not only barrier systems but also the cardiovascular and renal systems.

Histopathological and cellular changes

Mandour et al.⁷⁴ examined the effects of e-cigarette vapor on the submandibular gland of rats, showing significant histopathological changes such as vacuolated cytoplasm and thickened connective tissue. Oxidative stress markers, such as malondialdehyde (MDA), were elevated, indicating tissue damage, though some recovery was observed after cessation of e-cigarette exposure.

Therapeutic interventions and protective mechanisms

While the majority of studies highlighted the harmful effects of e-cigarettes, some research explored potential therapeutic interventions. Kadry et al.⁷⁵ investigated the protective effects of metformin on BBB integrity in iPSC-derived brain microvascular endothelial cells. The study found that metformin pre-treatment mitigated the permeability changes induced by e-cigarette vapor, preserving the expression of key tight junction proteins like ZO-1 and occludin. This suggests that certain pharmacological agents may offer protection against the barrier-disrupting effects of e-cigarettes, opening avenues for potential therapeutic strategies to combat e-cigarette-induced damage.

Sivandzade et al.⁷⁶ examined the protective effects of rosiglitazone on e-cigarette and tobacco smoke-induced BBB impairment. The study demonstrated that both e-cigarette vapor and traditional tobacco smoke disrupt BBB integrity by reducing tight junction proteins and increasing permeability. Rosiglitazone, a PPAR γ agonist, was shown to mitigate these effects, preserving BBB structure and reducing pro-inflammatory

cytokine levels. This highlights the potential for therapeutic interventions targeting inflammatory pathways to protect against e-cigarette-induced BBB damage.

Molecular and cellular mechanisms of e-cigarette exposure

The studies summarized in Table 3 elucidate the diverse molecular and cellular mechanisms by which e-cigarette exposure exerts its effects across various biological systems. Aboaziza et al.⁵⁸ demonstrated that e-cigarette vapor increases oxidative stress and reduces nitric oxide bioavailability, leading to arterial stiffness and endothelial dysfunction. Ahmad et al.⁵⁷ found that nicotine aerosol elevates inflammatory markers and disrupts the alveolar-capillary barrier, resulting in pulmonary edema and lung injury. Archie et al.⁶⁴ reported that e-cigarette vapor reduces the expression of tight junction proteins, compromising BBB integrity and impairing motor and cognitive functions. Burrage et al.⁵⁹ observed impaired endothelial-dependent dilation and increased extracellular vesicles in response to e-cigarette vapor, indicating cerebrovascular dysfunction. Chen et al.⁶⁵ highlighted the role of nicotine in enhancing *E. coli* invasion through actin cytoskeleton rearrangement and activation of PI3K/ROCK pathways.

Crotty Alexander et al.³¹ showed that e-cigarette vapor increases proinflammatory cytokines, reduces airway barrier function, and induces multiorgan fibrosis. Hasan et al.⁶⁶ linked e-cigarette aerosol and high-fat diet exposure to increased cardiomyocyte apoptosis, oxidative stress, and cardiac dysfunction. Heldt et al.⁴³ found that e-cigarette vapor increases leukocyte-endothelial interactions and microglial activation, leading to cognitive impairments. Kadry et al.⁷⁵ demonstrated that e-cigarette vapor increases BBB permeability and alters tight junction protein expression, with metformin providing protective effects. Kaisar et al.^{68,69} reported increased oxidative stress, BBB integrity impairment, and mitochondrial depolarization due to tobacco smoke and e-cigarette vapor, with metformin offering partial protection.

Lockman et al.⁶⁰ observed decreased BBB permeability and reduced brain uptake of MLA following chronic nicotine exposure. Mandour et al.⁷⁴ identified histopathological changes, inflammation, and oxidative damage in the submandibular gland due to e-cigarette vapor. Marinucci et al.⁷⁰ reported impaired Sertoli cell function and increased BTB permeability in response to nicotine. Mekala et al.^{67,72} highlighted mitochondrial dysfunction, increased EV release, and ER stress due to e-cigarette vapor, with P2 × 7 receptor antagonist providing protective effects. Orzabal et al.⁷¹ found that e-cigarette aerosols decrease fetal and pup growth and reduce blood flow in maternal and fetal arteries. Shah et al.⁶³ reported reduced glucose transport across the BBB and decreased GLUT1 expression due to nicotine pre-exposure. Sifat et al.^{61,62} observed decreased neuronal glucose uptake, reduced glycolytic capacity, and increased brain injury due to nicotine and e-cigarette vapor. Sivandzade et al.⁷⁶ demonstrated that RSG protects against BBB impairment caused by tobacco smoke and e-cigarette vapor. Finally, Zhang et al.⁷³ reported that nicotine induces ferroptosis, disrupts BTB integrity, and impairs sperm parameters. These findings collectively underscore the multifaceted adverse effects of e-cigarette exposure on various physiological systems.

Discussion

The studies included in this systematic review collectively reveal the wide-ranging and potentially harmful effects of e-cigarettes on human barrier systems. Whether through pulmonary inflammation, vascular dysfunction, or disruption of the blood-brain barrier, e-cigarettes significantly compromise the structural and functional integrity of these critical protective systems. The consistent findings of oxidative stress and inflammation as central mechanisms across various organs underscore the systemic risks posed by e-cigarettes, which extend beyond the lungs to affect the brain, heart, reproductive system, and more.

Importantly, the evidence of long-term developmental effects,

particularly in offspring exposed to e-cigarettes during pregnancy, highlights the need for public health interventions and regulatory scrutiny. Even in the absence of nicotine, e-cigarettes contain chemicals capable of inducing profound biological changes, with lasting consequences for cardiovascular, neurological, and reproductive health.

E-cigarette exposure disrupts airway epithelial barrier function by decreasing trans-epithelial electrical resistance and altering apical junctional complexes, exacerbating viral infections like RSV.⁴⁹ Both nicotine-containing and nicotine-free e-cigarette components compromise pulmonary endothelial integrity through ARF6-dependent pathways and oxidative stress.^{42,77} E-cigarette use also impairs gut barrier function, downregulating tight junction proteins and triggering inflammation.^{39,40} Chronic e-cigarette exposure disrupts blood-brain barrier integrity, promoting neuroinflammation and cognitive impairment.⁴³ These effects are partly independent of nicotine, with non-nicotine components like acrolein contributing to barrier disruption.⁴² Chronic e-cigarette inhalation induces systemic inflammation, multiorgan fibrosis, and cardiorenal dysfunction in mice.³¹

E-cigarette vapor induces oxidative stress and inflammation in pulmonary, cardiovascular, and neural systems through various mechanisms. The aerosol contains reactive oxygen species, including hydroxyl radicals, which can cause cellular damage.⁷⁸ E-cigarette exposure activates the phagocytic NADPH oxidase (NOX-2), leading to vascular dysfunction and oxidative stress.⁷⁹ The aerosol components, particularly flavoring additives and propylene glycol, increase inflammatory cytokine production and reduce antioxidant levels in lung cells and tissues.^{80,81} E-cigarette use is associated with endothelial dysfunction, increased risk of cardiovascular diseases, and potential neurodegenerative effects.^{82,83} The toxicity of e-cigarette aerosol is partly attributed to reactive aldehydes generated during aerosolization.^{81,84} Overall, e-cigarette vapor exposure can lead to oxidative stress-induced damage in multiple organ systems, raising significant public health concerns.⁸⁵ Chronic inhalation of e-cigarette vapor increases circulating pro-inflammatory and pro-fibrotic proteins, leading to organ damage and dysfunction.³¹ Studies have reported adverse effects on the pulmonary, cardiovascular, renal, and hepatic systems.^{86,87} E-cigarette aerosol exposure enhances oxidative stress and inflammation, potentially increasing the risk of organ dysfunction.⁸⁵ Biomarkers of inflammation, oxidative stress, and extracellular matrix breakdown have been observed in e-cigarette users.⁸⁸ Animal studies have shown increased arterial stiffness, vascular endothelial changes, and atherosclerotic plaque formation.⁸⁹ While e-cigarettes may produce lower levels of toxicants compared to conventional cigarettes, they still pose risks to multiple organ systems.²³ Long-term effects remain largely unknown, necessitating further research.⁹⁰

Research indicates that e-cigarette exposure can disrupt BBB integrity and promote neuroinflammation. Studies have shown that e-cigarette vapor and nicotine exposure can lead to downregulation of genes critical for BBB function, reduced expression of tight junction proteins, and increased BBB permeability.^{43,75} These effects are associated with oxidative stress, inflammation, and activation of matrix metalloproteinases.^{42,91} Nicotine specifically has been found to alter the distribution of tight junction proteins and increase BBB permeability.⁹² The impact of e-cigarette exposure extends to prenatal exposure, with studies showing long-term neurovascular changes and impaired behavioral outcomes in offspring.⁶⁴ Some protective effects have been observed with metformin and rosiglitazone, which may counteract the negative impacts of e-cigarette exposure on BBB integrity.^{75,76}

Nicotine exposure significantly alters glucose transport and energy metabolism in the brain. Chronic nicotine treatment increases glucose oxidation in glutamatergic neurons and enhances excitatory activity in most brain regions.⁹³ However, nicotine pre-exposure reduces glucose transporter-1 (GLUT1) function and expression at the blood-brain barrier, potentially creating a glucose-deprived state in the neurovascular unit.⁶³ This effect is exacerbated when combined with oral contraceptives, leading to altered glycolysis and increased ischemic brain

Table 3
Summary of molecular and cellular mechanisms of e-cigarette exposure effects.

Reference	Mechanisms	Proposed mechanistic pathway
Aboaziza et al. 2023 ⁵⁸	Increased oxidative stress, reduced nitric oxide bioavailability, structural changes in the aorta (reduced elastin, increased collagen).	E-cigarette vapor → Oxidative stress ↑ → Nitric oxide ↓ → Arterial stiffness ↑, Endothelial dysfunction ↑
Ahmad et al. 2019 ⁵⁷	Increased inflammatory markers (IL-1A, CXCL1, MPO), disruption of the alveolar-capillary barrier.	Nicotine aerosol → Inflammatory markers ↑ → Alveolar-capillary barrier disruption → Pulmonary edema ↑, Lung injury ↑
Archie et al. 2023 ⁵⁴	Reduced expression of tight junction proteins (ZO-1, claudin-5, occludin), impaired motor and cognitive functions.	E-cigarette vapor → Tight junction proteins ↓ → BBB integrity ↓ → Motor and cognitive impairments ↑
Burrage et al. 2021 ⁵⁹	Impaired endothelial-dependent dilation, increased extracellular vesicles.	E-cigarette vapor → Endothelial-dependent dilation ↓ → Cerebrovascular dysfunction ↑
Chen et al. 2002 ⁶⁵	Enhanced E. coli invasion, actin cytoskeleton rearrangement, PI3 K and ROCK signaling pathways.	Nicotine → Actin cytoskeleton rearrangement ↑ → E. coli invasion ↑ → PI3K/ROCK pathways activation
Crotty Alexander et al. 2018 ^{31,43,60–63,66–76,129,130}	Increased proinflammatory cytokines, reduced airway barrier function, multiorgan fibrosis.	E-cigarette vapor → Proinflammatory cytokines ↑ → Airway barrier function ↓ → Systemic inflammation ↑, Multiorgan fibrosis ↑
Hasan et al. 2021 ⁶⁶	Increased cardiomyocyte apoptosis, oxidative stress, ultrastructural abnormalities in the heart.	E-cigarette aerosol + HFD → Cardiomyocyte apoptosis ↑ → Oxidative stress ↑ → Cardiac dysfunction ↑
Heldt et al. 2020 ⁴³	Increased leukocyte-endothelial interactions, microglial activation, cognitive impairments.	E-cigarette vapor → Leukocyte-endothelial interactions ↑ → Microglial activation ↑ → Cognitive impairments ↑
Kadry et al. 2021 ⁷⁵	Increased BBB permeability, altered tight junction protein expression, protective effects of metformin.	E-cigarette vapor → BBB permeability ↑ → Tight junction proteins ↓ → Metformin → BBB integrity protection
Kaisar et al. 2017 ⁶⁸	Increased oxidative stress, BBB integrity impairment, inflammation, stroke injury, protective effects of metformin.	Tobacco smoke/e-cigarette vapor → Oxidative stress ↑ → BBB integrity ↓ → Inflammation ↑ → Stroke injury ↑ → Metformin → Protection
Kaisar et al. 2018 ⁶⁹	Mitochondrial depolarization, altered iron transporters, differential gender responses.	Tobacco smoke/e-cigarette vapor → Mitochondrial depolarization ↑ → Iron transporters (Slc40a1, Abcb6) ↑ → Gender-specific responses
Lockman et al. 2005 ⁶⁰	Decreased BBB permeability, reduced brain uptake of MLA.	Chronic nicotine exposure → BBB permeability ↓ → Brain uptake of MLA ↓
Mandour et al. 2021 ⁷⁴	Histopathological changes in the submandibular gland, inflammation, oxidative damage.	E-cigarette vapor → Histopathological changes ↑ → Inflammation (TNF-α) ↑ → Oxidative damage (MDA, GPx1) ↑
Marinucci et al. 2020 ⁷⁰	Impaired Sertoli cell function, altered ECM and tight junction protein expression, increased BTB permeability.	Nicotine → Sertoli cell function ↓ → ECM and tight junction proteins ↓ → BTB permeability ↑
Mekala et al. 2022 ⁶⁷	Reduced mitochondrial OXPHOS, increased intracellular calcium, ER stress.	E-cigarette vapor → Mitochondrial OXPHOS ↓ → Intracellular calcium ↑ → ER stress ↑
Mekala et al. 2024 ⁷²	Mitochondrial dysfunction, increased EV release, paracrine signaling, protective role of P2 × 7 receptor antagonist.	E-cigarette vapor → Mitochondrial dysfunction ↑ → EV release ↑ → Paracrine signaling ↑ → P2 × 7 receptor antagonist → Protection
Orzabal et al. 2019 ⁷¹	Decreased fetal and pup growth, reduced blood flow in maternal and fetal arteries.	E-cigarette aerosols (nicotine) → Fetal/pup growth ↓ → Blood flow in maternal/fetal arteries ↓
Shah et al. 2015 ⁶³	Reduced glucose transport across BBB, decreased GLUT1 expression.	Nicotine pre-exposure → Glucose transport across BBB ↓ → GLUT1 expression ↓
Sifat et al. 2018 ⁶¹	Decreased neuronal glucose uptake, reduced glycolytic capacity, mitochondrial respiration.	Nicotine/e-cigarette vapor → Neuronal glucose uptake ↓ → Glycolytic capacity ↓ → Mitochondrial respiration ↓
Sifat et al. 2022 ⁶²	Increased brain injury, reduced BBB tight junction proteins, increased inflammatory and oxidative stress markers.	JUUL e-cigarettes/tobacco smoke → Brain injury ↑ → BBB tight junction proteins ↓ → Inflammatory/oxidative stress markers ↑
Sivandzade et al. 2019 ⁷⁶	Reduced BBB integrity, increased oxidative stress, inflammation, protective effects of RSG.	Tobacco smoke/e-cigarette vapor → BBB integrity ↓ → Oxidative stress ↑ → Inflammation ↑ → RSG → Protection
Zhang et al. 2023 ⁷³	Induced ferroptosis, disrupted BTB integrity, impaired sperm parameters.	Nicotine → Ferroptosis ↑ → BTB integrity ↓ → Sperm parameters ↓

damage.⁹⁴ Nicotine exposure decreases brain glucose utilization in ischemic conditions, downregulating GLUT1 and GLUT3 expressions.⁶¹ Moreover, prenatal nicotine exposure affects glial development and neurometabolite levels in young children.⁹⁵ These alterations in glucose metabolism and energy homeostasis contribute to nicotine’s effects on appetite regulation and body weight.⁹⁶

Chronic e-cigarette exposure has been shown to negatively impact reproductive health and blood-testis barrier (BTB) integrity. Studies in animal models reveal that e-cigarette liquid contains harmful chemicals that can impair antioxidant defenses, cause degenerative changes in testicular tissue, and disrupt the BTB.^{97,98} E-cigarette exposure leads to reduced testosterone levels, decreased sperm quality, and increased oxidative stress in the testes.^{97,99} The BTB, crucial for spermatogenesis, is particularly vulnerable to e-cigarette-induced damage, with studies showing decreased expression of tight junction, adherens junction, and gap junction proteins.^{100,101} These effects are similar to those observed with exposure to fine particulate matter (PM_{2.5}) and chronic stress, both of which also compromise BTB integrity and sperm parameters.^{101,102} Overall, the evidence suggests that e-cigarettes cannot be considered a safe alternative to conventional cigarettes, particularly concerning reproductive health.¹⁰³

Research suggests that maternal e-cigarette exposure during pregnancy may have long-term cardiovascular effects on offspring. Studies in

rats have shown that maternal vaping, with or without nicotine, leads to persistent vascular dysfunction in offspring, including increased arterial stiffness and impaired aortic relaxation.^{58,59} These effects were observed in both adolescent and adult offspring. Chronic e-cigarette exposure in mice has been found to impair cardiovascular function, including increased arterial stiffness and altered cardiac function.^{104,105} The severity of cardiovascular dysfunction increases with exposure duration and nicotine content.¹⁰⁵ While e-cigarettes are often promoted as a safer alternative to traditional cigarettes, current evidence suggests that they are not harm-free and may pose significant cardiovascular health risks.^{106,107} Further research is needed to fully understand the long-term cardiovascular effects of maternal e-cigarette exposure on offspring.

E-cigarette use during pregnancy poses potential risks to fetal and neonatal development. Studies have linked prenatal e-cigarette exposure to low birth weight, preterm birth, and small-for-gestational-age infants.¹⁰⁸ Animal research indicates that e-cigarette aerosols can disrupt fetal lung development, leading to altered gene expression and respiratory function.¹⁰⁹ Nicotine from e-cigarettes can accumulate in fetal blood, potentially affecting multiple organ systems.¹¹⁰ Neuro-behavioral assessments reveal that e-cigarette-exposed infants exhibit abnormal reflexes and decreased motor maturity.¹¹¹ DNA methylation changes and reduced neural cell viability have been observed in animal studies.¹¹² Neonatal mice exposed to e-cigarette emissions showed

impaired weight gain and lung growth.¹¹³ While some perceive e-cigarettes as safer than traditional cigarettes during pregnancy, current evidence suggests potential harm to fetal development.^{114,115}

The systemic effects of e-cigarettes on various human barrier systems raise critical public health concerns, particularly given the widespread perception that they are a safer alternative to traditional tobacco products.¹¹⁶ Emerging evidence indicates that e-cigarette use can lead to significant disruptions in the pulmonary, cardiovascular, reproductive, and neural barrier systems, resulting in long-term health risks such as cardiovascular disease, neuroinflammation, and multi-organ fibrosis. The potential for intrauterine growth restriction and developmental abnormalities in offspring due to maternal e-cigarette exposure further underscores the urgent need for regulatory measures to limit their use, especially among vulnerable populations.

Recent research highlights the complex landscape of e-cigarette policy and public health recommendations. While some view e-cigarettes as a harm reduction tool for smokers, others express concerns about youth initiation and potential health risks.^{117,118} Evidence supporting policy recommendations often contains conflicts of interest, including relationships with tobacco and e-cigarette industries.¹¹⁹ For pregnant women and adolescents, e-cigarette use poses particular concerns due to nicotine's developmental effects.^{120,121} Adolescent e-cigarette use may increase the risk of cigarette smoking during pregnancy.¹²² Public health policies must balance the potential benefits for adult smokers with the need to protect vulnerable populations.¹²³ Given the limited long-term data and mixed evidence, further research is needed to fully assess the risks and benefits of e-cigarettes, particularly for youth and pregnant women.¹²⁴

Research on e-cigarettes reveals a complex landscape with potential benefits and risks. While e-cigarettes deliver lower levels of carcinogens than traditional cigarettes, they still expose users to toxins that may increase cardiovascular and lung disease risks.¹²⁵ Some studies suggest e-cigarettes could aid in smoking cessation, but evidence is limited.¹ Concerns exist about e-cigarettes attracting youth and potentially leading to traditional cigarette use.¹²⁶ The rapid evolution of e-cigarette products complicates research efforts.¹ There is a need for standardized measurements of e-cigarette contents and emissions, as well as studies on their physiological effects and patterns of use.²⁹ While e-cigarettes may offer harm reduction potential, more research is needed to fully understand their impact on public health.¹²⁷ Researchers caution against conceptualizing e-cigarettes as equivalent to traditional cigarettes.¹²⁸

Health promotion efforts should also target smoking cessation programs, incorporating education about the risks of e-cigarette use. Special focus should be given to pregnant women, emphasizing the risks of e-cigarette use during pregnancy and its potential long-term effects on fetal development. Integrating these concerns into maternal healthcare guidelines and public health policy will be essential for mitigating the growing health burden associated with e-cigarette use.

Conclusions

This review provides robust evidence of the harmful effects of e-cigarette exposure on human barrier systems. Through mechanisms such as oxidative stress and inflammation, e-cigarettes compromise the integrity of pulmonary, vascular, and neural barriers, leading to long-term health risks. These findings underscore the urgent need for public health policies to regulate e-cigarette use, particularly among adolescents, pregnant women, and other vulnerable populations. Further research is needed to fully understand the chronic effects of e-cigarette exposure and to develop safer alternatives.

CRediT authorship contribution statement

Gabriella Lupo: Writing – review & editing, Validation. **Carmelina Daniela Anfuso:** Writing – review & editing, Investigation,

Conceptualization. **Giuseppe Smecca:** Validation, Investigation. **Alessia Cosentino:** Investigation. **Aleksandra Agafonova:** Formal analysis. **Chiara Prinzi:** Formal analysis. **Rosario Junior Ferrauto:** Formal analysis. **Stefano Turzo:** Formal analysis. **Venerando Rapisarda:** Formal analysis. **Caterina Ledda:** Writing – original draft, Investigation, Data curation, Conceptualization.

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