Detection and Quantitative Determination of Heavy Metals in Electronic Cigarette (Vaping) Refill Liquids

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Abstract

Electronic cigarettes (E-cigarettes) are a smoking cessation option for traditional cigarette smokers. It gained popularity among current smokers and former non-smokers, according to current knowledge. Research at various levels showed that e-cigarettes are dangerous. This review offers a summary of E-cigarettes toxicity based on current in vivo and in vitro studies. We also go through the toxicity components found in e-cigarettes, as well as the possible mechanism by which e-cigarettes cause damage. Heavy metals in conventional cigarettes and e-cigarettes have a certain level of toxicity, as we all know. Metals like nickel, cadmium, lead, zinc, aluminum, chromium, manganese and tin, have been reported to be present in e-cigarette aerosols, dispenser, and/or e-liquid A few studies have also found that the propylene glycol and vegetable glycerin mixture, as well as flavoring agents in e-cigarettes, are other components that cause negative effects in animals or cells.

1.Introduction

Tobacco smoking is the most preventable cause of morbidity and mortality. In just a few short years, electronic cigarettes (E-cigarettes) have become increasingly popular, especially for younger individual many people believe that E-cigarettes are safe. The inhaled aerosols of E-cigarettes contain numerous potential toxicities, some of which could be dangerous for health with long-term use (Miler and Hajek, 2018).

Electronic nicotine delivery systems (ENDS), commonly known as e-cigarette is a battery-powered device that contains aerosolized nicotine delivered to its users in the form of vapor instead of smoke, the battery-operated devices generate aerosols by heating a liquid solution with a metal coil. Electronic cigarette (e-cigarette) use is increasing worldwide, yet, relatively little is known about its long-term health effects. Few studies have evaluated the e-cigarette device itself, and whether the e-liquid changes once it comes into contact with the device (Badea et al. , 2018) . The heating coils, for instance, are primarily made up of metals. Once the coil is heated, the e-liquid is aerosolized and inhaled by the user. In a recent study, metal concentrations in the aerosol and e-liquid from the tank were markedly higher compared to e-liquid from the refilling dispenser, demonstrating metals were transferred from the coil to the liquid in the tank and the aerosol (Bansal and Kim 2016).

Contamination by heavy metals and, more recently, by rare earth elements (REE) and other minor elements (ME), has increased during the last decades due in part to their high use in technological and electronic devices. Although some heavy metals are necessary for life, most are considered non-essential and some have adverse health effects to humans—and other vertebrates—even at very low concentrations **(Tchounwou et al., 2012)**. Moreover, some essential elements are included in the ATSDR's (Agency for Toxic Substances and Disease Registry) priority pollutant list for being toxic to living organisms at high concentrations. Thus, a total of 23 elements are included in the ATSDR's priority pollutants list: silver (Ag), aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), palladium (Pd), plutonium (Pu), antimony (Sb), selenium (Se), strontium (Sr), thallium (Tl), thorium (Th), uranium (U), vanadium (V), and zinc (Zn) **(Flanagan et al., 2018)**.

2.The history of Electronic Cigarettes

The first electronic cigarette was created in 1963 when an American engineer named Herbert A. Gilbert filed a patent for a device that produced a nicotine-containing steam **(Mikheev et al.,2016)**. However, this device was never commercialized. The modern electronic cigarette was invented in 2003 by a Chinese pharmacist named Hon Lik for his father who was a heavy smoker with lung cancer. E-cigarettes were sold first in China in 2004 and later exported by the Ruyan company and made available over the Internet and more recently in retail establishments in Europe and in the United States **(Rice et al., 2001)**. E-cigarettes heat and vaporize a solution containing nicotine, and many are designed to look outwardly like traditional tobacco cigarettes. Thus, as a cigarette-like device that mimics both hand-to-mouth and oral-sensory experiences of a traditional cigarette, E-cigarettes have the potential to attract significant numbers of customers who might otherwise smoke cigarettes **(Gaur and Agnihotri, 2019)**.

In the last few years, major tobacco companies such as Lorillard, British American Tobacco, Altria, Reynolds and Imperial Tobacco have launched their own EC brands and are buying up existing ones. Marketing and sale have exploded and EC-shops and -lounges pop-up everywhere. For the first time in more than 40 years tobacco companies are back on TV with cigarette ads CNN Money, 2014. Industrial economists project that the ECs will surpass conventional cigarettes (CC) in about three decades, and the global EC market is expected to hit $10 billion by 2017 **(Visser et al., 2019)**.

3.Demographic characteristics of E-cigarette use

To date, data from 5 poison control center databases have been published. Between September 2010 and February 2014, the number of e-cigarette exposures in all reports was 2405 total calls with calls increasing from 1 per month to 215 per month. Forty-two percent of the calls involved children younger than 5 years, and 27.4% involved adults aged 20 to 39 years. The majority of the calls concerned accidental ingestion of e-liquid in children and reported relatively mild toxicity **(Orellana-Barrios, 2015)**.

E-cigarettes have gained popularity among youth and young adults due to the increased awareness of the conventional cigarettes' hazards, health authorities' legislation, and restrictions on conventional cigarettes smoking in public places, continues focused marketing campaigns by the manufacturers and popular perception of E-cigarettes as a healthier alternative than conventional cigarette **(Visser et al., 2019)**.

3.1.Here are some reasons that youth may use E-cigarettes:

-They are curious.
-They like the flavors they come in.
-They think they are safe.
-They seek the stimulant quality of nicotine.
-They want to perform tricks with the exhaled vapor.
-They want to push limits set by adults.
-Their friends are using.
-Exposure to e-cigarette marketing

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4.Types of Electronic cigarettes

There are several different types of E-cigarettes, including Vape Pen, Box Mod, Mech Mod, Pod Mod, HnB and E-hookah, etc.

-Vape Pen is a pen type e-cigarette.

-Box Mod is a box shaped e-cigarette, named after its shape like a box.

-Mech Mod is a mechanical smoke, an e-cigarette device that does not contain a control chip, and its safety depends on the knowledge of the player.

-Pod Mod is a closed e-cigarette with replaceable cartridges.

-HnB is a heat-not burn smoking device.

-E-hookah is an electronic hookah.

5.Composition of E-cigarettes

E-cigarettes has three basic components: a battery, a heating element, and a liquid-containing cartridge. The secondary parts include an airflow sensor, a microchip for controlling the heating element, and a light-emitting diode at the tip that simulates a burning cigarette. The air holes in the devices control the pressure drop and facilitate the air flow required for puffing. The batteries in ECs may be activated automatically through inhalation or manually by pressing the activating button **(Brown and Cheng , 2014)**.

The structure of ECs (Figure1) has undergone tremendous change since their introduction in 2003. This includes changes in their size, nicotine concentrations, atomizer, e-liquid composition, and the type of batteries. There are three generations of ECs. The second- and third-generation devices allow user customization, which influences aerosol production, nicotine delivery, and risk associated with the use of the product **(Farsalinos and Polosa, 2014)**.

5.1.Atomizer

The atomizer is a chamber consisting of a resistance wire encircling the wicking device that draws in the fluid. The smoke is generated through the activation of atomizer by the sensing device. Subsequently, the resistance wire is heated up, and e-liquid vapors are released, which are inhaled by the user. This part of ECs has undergone numerous modifications resulting in the development of "cartomizers" (cartridge plus atomizer), which are a combination of e-liquid distribution system, the wick, and heating element **(Hua and Talbot,2016)**.

The quantity and quality of aerosol, including the concentration of metals, is dependent on the material of the heating element, its inherent resistance, voltage applied, and temperature. Warmer air can hold greater quantity of e-liquid per unit volume. The heating element is customarily made of nichrome (an alloy of Ni and Cr) or a combination of nichrome and Kanthal (an alloy of Fe, Cr, and Al). Therefore, Ni and Cr are most often leached from this unit. Additionally, metals like Cu, Ag, Zn, Sn, Pb, Ca, Mg, Fe, and Al may also be released from the other components of ECs. Their detailed analysis is discussed in a later section **(Flanagan et al., 2018)**.

5.2.Batteries

The ECs may be powered by a permanent rechargeable battery or a non-rechargeable battery or a user-replaceable battery. These batteries include metals like Ni-Cd, Ni metal-hydride, lithium (Li) alkaline, Li-polymer, and Li-Mn. These metals may leach into the aerosol from batteries **(Badea et al. , 2018)**.

5.3.E-Liquid

The cartridge can be filled with different e-liquid solutions. They are vaporized by the heating element, and the cigarette is consumed by inhaling the aerosol mist. The most common chemicals used are propylene glycol and vegetable or aqueous glycerin, in the ratio of 4:1 **(Prokopowicz et al., 2019)**.

Besides, flavors are added to improve the taste of the liquid. Nicotine is added to propylene glycol in concentrations up to 70 mg/mL . Although propylene glycol is a safe, colorless, and odorless food additive, nicotine is very harmful to both general and oral health **(Lee et al.,2018)**.

Metals like Cd, Cr, Pb, Mn, and Ni have been identified in the e-liquids. The nicotine in e-liquids is derived from *Nicotiana tabacum* (cultivated tobacco), a potent bio-accumulator. It absorbs pollutants including the heavy

metals from the immediate growing environment. Therefore, Pb may be introduced into the e-liquids during the extraction of nicotine from tobacco leaves **(Carnevale** *et al.,***2016)**. Furthermore, heating coil, wick, and other internal components of ECs may introduce Pb into the e-liquid **(Jain ,2019)**.

5.4.Mouthpiece

It is the piece the person uses to inhale a tobacco pipe containing a nicotine solution, an evaporation device, a battery, and a mouthpiece.
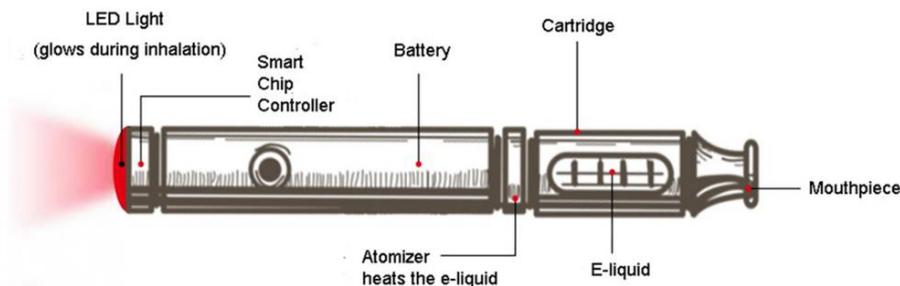

Figure (1):Structure of E-cigarrete **(Carnevale** *et al.,***2016)**

The e-cigarette aerosol that users breathe from the device and exhale can contain harmful and potentially harmful substances, including:

- Nicotine.
- Ultrafine particles that can be inhaled deep into the lungs.
- Flavoring such as diacetyl, a chemical linked to a serious lung disease.
- Volatile organic compounds.
- Cancer-causing chemicals.
- Heavy metals such as nickel, tin, and lead

6.Origins of e-cigarettes toxicity

Smoking is considered an important source for inorganic elements intake, mainly for some trace elements and other biochemically important elements. Vapours' toxicant intake varies depending of which different e-liquids are used, the type of vaporizers, battery power settings and vaping regimes. In that sense, formaldehyde, acetaldehyde, acrolein, diacetyl, acetol, glycidol, nicotine, nicotyrine, acenaphthene, isovaleraldehyde, formaldehyde, benzaldehyde and benzene have been detected in the vapour of e-cigarettes. However, other chemicals are not directly present in the e-liquids, but are either released from hardware components of the e-cigarette such as metal and silicate particles . It has been demonstrated that increasing battery outputs generates also increasing levels of some residues such as carbonyls **(Prokopowicz et al., 2019)**.

6.1.Nicotine

Nicotine , also called 3-(1-methyl-2-pyrrolidinyl)pyridine according to the IUPAC a nomenclature, is a bicyclic compound with a pyridine cycle and a pyrimidine cycle (Figure 2) .The molecule possesses an asymmetric carbon and so exists in two enantiomeric compounds. Nicotine is one of the most important components of the solution in electronic cigarettes with different concentrations **(Madison** *et al.,* **2020)**.
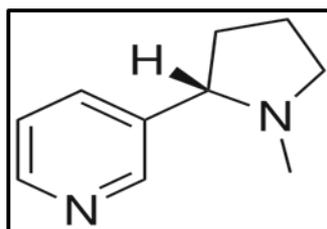

Figure (2):Structure of Nicotine molecule **(Wagener** *et al.,* 2017)

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The concentration of nicotine in e-cigarettes in most cases, ranges from 0 mg/ml to 36 mg/ml. The amount of nicotine absorbed is affected by the device and the amount of e-liquids vaporized **(Wagener et al., 2017)**. However, there is no doubt that in addition to being addictive, nicotine has a certain negative impact on health. For example, **Schroeder and Hoffman (2014)**. showed that smoking an electronic cigarette with a 30s interval for 10 times, the heart rate and serum nicotine concentration increased significantly in 5 min, indicating that nicotine in e-cigarettes is rapidly absorbed. In addition, **Wu et al (2014)** exposed primary human airway epithelial cells to tobacco-flavored e-liquid with or without nicotine, and found that nicotine-free e-liquid promoted proinflammatory levels, whereas nicotine-containing e-liquid elevated the effect.

The rate of nicotine metabolism been to vary by sex and race, which may influence susceptibility to addiction and ability to quit. Women metabolize nicotine faster than men, which may explain why women have more difficulty in quitting. Nicotine is mainly metabolized by the liver (85%–90%), and the metabolites are then excreted through the kidneys. Only 10% of nicotine is excreted unchanged.

6.1.1.Nicotine exposure in pregnancy

Nicotine adversely affects maternal and foetal health during pregnancy, contributing poor outcomes including preterm delivery, still birth, neonatal apnoea, and sudden infant death syndrome **(England et al.,2015)**. Nicotine exposure during pregnancy also has lasting adverse consequences for brain and lung development. There is currently no clinical knowledge of the efficacy and safety of e-cigarette use in pregnancy, but no amount of nicotine is known to be safe during pregnancy. Therefore, nicotine delivered by E-cigarettes during pregnancy can result in multiple adverse consequences, including sudden infant death syndrome, and could result in altered corpus callosum, deficits in auditory processing, and obesity **(Suter et al., 2015)**.

6.1.2.Nicotine exposure in adolescence

There is evidence that adolescents who are exposed to nicotine may become addicted more rapidly, and at lower or more intermittent levels of consumption than adults. Evidence suggests that nicotine exposure during adolescence, a time during which the brain undergoes rapid development, may have a long-term negative impact on higher cognitive function **(Lippi et al., 2014)**.

6.2.Metals

Attention has been paid to analyze constituents of e-liquids. Metals like nickel, cadmium, lead, zinc, aluminum, chromium, manganese, iron, antimony, tin, and arsenic have been reported to be present in e-cigarette aerosols, dispenser, and/or e-liquid **(Olmedo et al., 2018)**.

Numerous studies, moreover, have measured elevated levels of toxic organic and inorganic chemicals in e-cigarette liquid and aerosols **(Dunbar et al., 2018; Kamilari et al., 2018; Palazzolo et al., 2017)** .The presence of metals and metalloids (e.g., arsenic, chromium, lead, nickel) in e-cigarette aerosols is a major concern, given their serious health effects, including cancer, cardiovascular disease **(Song et al., 2018)**, renal damage, and neurotoxicity. Metals/metalloids may originate from the coil and from soldered joints and other parts of the device **(Williams and Talbot 2019)**. Commonly used coils are made of alloys (e.g., kanthal [iron (Fe), chromium (Cr), and aluminum (Al)], nichrome [nickel (Ni) and Cr], or high-purity metals (e.g., Ni or titanium)) **(Olmedo et al., 2018)**. Tin (Sn) and other metals are used in soldered joints. E-liquids may also contain arsenic (As) and other metals/metalloids at varying levels **(Zhao et al. ,2019)**.

In a cross-sectional study of 150 nonsmokers, cigarette smokers, and e-cigarette smokers, **Badea et al (2018)** reported cigarette smokers to have the highest levels of copper, molybdenum, zinc, antimony, and strontium and e-cigarette users to have the highest levels of selenium, silver, and vanadium in blood. Possible exposure to toxic and other metals from E-cigarettes generated aerosol and other components has also been investigated by **Palazzolo et al., (2017)**. **Williams et al (2015)** reported the presence of tin, copper, zinc, silver, nickel, and chromium in the aerosols of cartomizers of four different brands of E-cigarettes. Moreover , it was recorded that , mineral concentration is higher in the aerosolized e-liquids as compared to the non-aerosolized forms. Moreover, the concentration of some of these metals was higher in EC aerosol when compared to conventional tobacco smoke **(Williams et al.,2017)**. For

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instance, the levels of Ni and Cr have been found to be very high in aerosols due to their leaching from the core assembly. It was proposed that these metals could be trapped in respiratory tissues and could induce pathologies. Additionally, their increased concentration in body fluids may induce systemic and oral conditions **(Schober *et al.*,2014)**.

6.2.1.Detecting metals in samples

To demonstrate internal exposure to toxic metals associated with e-cigarette use, metal/metalloid levels have not only been measured in e-liquids and aerosols but also in human bio-samples such as urine **(Aherrera *et al.*, 2017; Goniewicz *et al.*, 2014; Jain 2019)**, saliva, serum **(Badea *et al.*, 2018)**, and blood collected from e-cigarette users. Thus, while cadmium or cupper are highly concentrated in cigarettes, for other elements—i.e. selenium—this association is inverted or even irrelevant (that is the case of mercury). Anyhow, cigarette smoking interferes with the carefully controlled metal homeostasis of the human body and has to be considered as harmful to health.

6.2.2.Source of Metals in Electronic Cigarettes

As shown in Figure 3, there are different sources of metals in aerosols from ECs. One study investigated metal substrates in different components of atomizer **(Lerner *et al.*, 2015)**. It was reported that metals like Ni and Cr were released from the nichrome filament, Cu and Ag from the thick wire, Cu and Zn from the brass clamp, Sn and Pb from solder joints, and Si, oxygen, Ca, Mg, and Al from the wick and sheath. The elemental analysis of the core assembly revealed the presence of trace metals, especially Al, Fe, Ni, and Zn. EC batteries and cartomizer were reported as sources of Cu in one study **(Fowles *et al.*, 2020)**.

An earlier detailed analysis of the core assembly by the same authors revealed that the inner and outer surfaces of the casing comprised of Fe and Mn. The core tip consisted of Ni, Cu, and Zn. The upper core was composed of Si while Zn and Pb were present in the gasket. The fabric material contained high percentages of Cu and Ni. The outer and inner surfaces of the woven tube comprised primarily of Si, Sn, and Al **(Staudt *et al.*, 2018)**. The upper and lower halves of the core were coated with Ag, with underlying metal compositions of Ni and Cu. The wick fibers within the surrounding resistance coil consisted mainly of Si while the coil filament around the wick fibers contained high quantities of Ni, with less amounts of Si and Mn. The weld joint connecting the coil with the thick extension wire was made up of high amounts of Ni and some Si. The thick extension wire beyond the weld joint consisted of Ni with minimal amounts of Cu. The juncture of thick extension wire, coil, and weld joint contained mainly Ni (53%) and Cr (18%)**(Aherrera *et al.*, 2017)**.
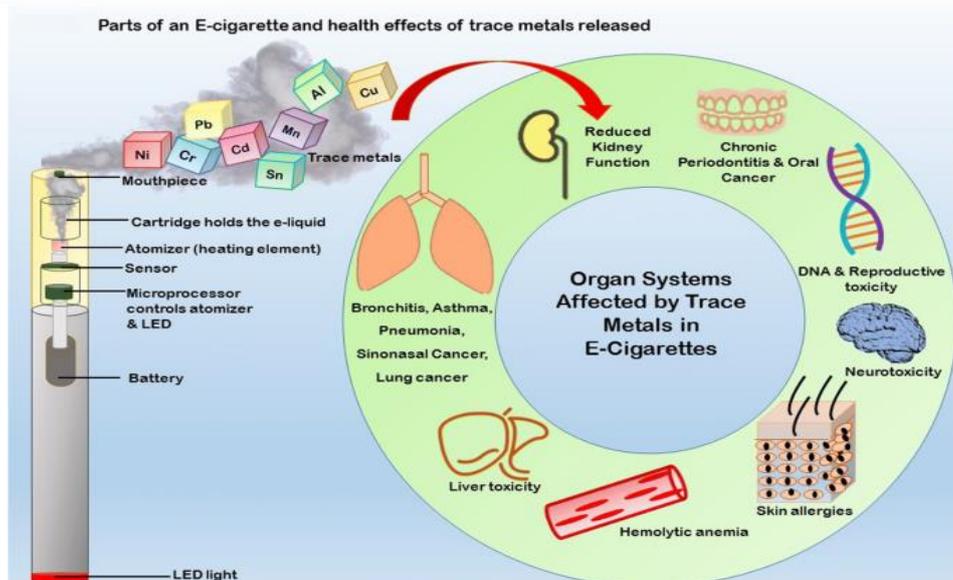

Figure (3):Parts of an e-cigarette and health effects of trace metals released **(Gaur and Agnihotri, 2019)**.

6.2.3.Genral health effects of Metals in E-cigarettes
6.2.3.1.Aluminum

Aluminum is highly concentrated in cigarettes, with values ranging from 699 – 1200 mg/g. This level can be easily reached in smoking rooms or rooms with poor ventilation. After inhalation, Al accumulates in the kidneys, brain, lungs, liver, and thyroid. It competes with Ca for absorption and affects the skeletal mineralization. In non Al-exposed individuals, a mean plasma concentration of 4.2 mg/l was found, but these levels were not influenced by age or smoking habits. Although the mechanism is not known, various data provides evidence that Al is associated with Alzheimer's disease (AD). Trace metal homeostasis plays a big role in the normal functioning of the brain, and disturbances in it can exacerbate events associated with AD . There is no hint, however, that smoking is directly related with AD. In addition, Al also plays a causal role in the development of microcytic anaemia and osteomalacia and can potentiate inflammatory and oxidative events **(Saffari et al., 2014)**. A study evaluated indoor levels of Al due to EC vaping. It involved six vaping sessions by nine volunteers who consumed ECs in a thoroughly ventilated room for 2 h. The authors reported a 2.4-fold increase in the concentration of Al after 2 h of vaping **(Goniewicz et al., 2014)**. Al whether actively (drawn) or passively inhaled accumulates significantly in surrogate lung fluids and causes respiratory, neurological, and other smoking-related diseases.

6.2.3.2.Cadmiun

Cigarette smoke is one of the major sources of airborne Cd , also ,Cadmium is the best studied metal from cigarette smoke, and smoking is the main source of cadmium intake by humans. The Cd amounts varied, the average Cd content per cigarette lies between 0.5 and 1.5 mg per cigarette . Smokers have twice the amount of Cd in their bodies as compared to non-smokers **(Cantrell, 2014)**. When the cigarette is smoked, Cd is transformed to cadmium oxide, which is then inhaled. Approximately 40 to 60% of the Cd inhaled from cigarette smoke passes through the lungs and body and 20 – 50% is transferred to the circulation. Therefore, for every 20 cigarettes smoked, approximately 2–4 μg of Cd is inhaled by the smoker and approximately a microgram spreads into the environment **(Wiener and Bhandari , 2020)**.

Mortada *et al* **(2004)** reported that the mean whole blood Cd content in smokers was 1.9 times higher than in non-smokers. **Satarug and Moore (2004)** reported that smokers have a 1.7 fold increase of serum Cd content compared to non-smokers. These data highlight the dramatic difference in serum cadmium levels between smokers and non-smokers at a young age, and that this difference is reduced but still present at older ages. Additionally, the accumulation of cadmium in the circulation with time can be seen.

Cadmium not only accumulates in the circulation but also in the kidneys, mainly in the kidney cortex. Several studies have shown that this accumulation of Cd in kidneys causes tubular dysfunction and renal end stage failure **(Prokopowicz et al., 2019)**. Its health effects include alteration of immune response at very low concentrations (0.1–10 μM). Cd inhibits the production of IgE in a concentration-dependent manner **(Ashraf ,2012)**. Further, an exposure for more than 24 h diminishes the activated B cells paralleled by a concomitant decrease in their viability and proliferation. Therefore, Cd may be both immunotoxic and immunomodulatory **(Olmedo et al.,2018)** . Further, Cd has been shown to reduce bone mineral density (osteoporosis) and to cause osteomalacia. Additionally, cigarette smoke delivered Cd has been shown to reduce birth weight, and although the placenta seems to protect the fetus from maternal Cd, child Cd burden increases soon after birth via breast milk. Finally, Cd is under suspicion to cause cataract, emphysema, hypertension, and cardiovascular disease **(Lee et al.,2018)**.

6.2.3.3.Chromium

Chromium (III) is the dominating species in the environment and may be an essential element by ingestion. Chromium (VI) is highly toxic and rapidly reduced to chromium (III) in the lung and intestinal tract. The main source of chromium (VI) is air pollution and water pollution from industrial sources, smoking and occupational settings. Cr concentrations are generally highest in lung tissue, where the concentration tends to increase with age. Chromium is excreted through the urine and feces (predominantly through the urine) **(Fadrowski et al., 2010)**.

The presence of Cr in EC smoke, although not widely interpreted, is a health concern. Acute effects of chromium toxicity include shortness of breath, coughing, wheezing, gastrointestinal effects (abdominal pain, vomiting, hemorrhage); chronic non-cancer effects include perforations and ulcerations of the nasal septum, bronchitis, decreased pulmonary function, pneumonia, asthma, nasal itching and soreness cancer effects include lung, nasal, and sinus cancer (**Aherrera** *et al.*, **2017**).

There was a very wide range of Cr in EC aerosol as reported in the studies included in the present review. The levels of Cr in mainstream cigarette smoke range from 0.0002 to 0.5 mg/cigarette. In smokers, its concentration in the lung tissue may be about 4.3 mg/kg (dry weight) as compared to 1.3 mg/kg in non-smokers. Accumulation of Cr in the lung tissue has been directly correlated with duration of smoking, age, and smoking time (**Aherrera** *et al.*,**2017**). Its concentrations have been reported to be significantly higher in all five lobes of smokers' lungs when compared to non-smokers. Chromium (VI) compounds, mostly hydroxyl radicals, induce DNA-damage (single strand breaks) and have potential cell transforming effects. Other, non cancerous, effects of Cr on the respiratory tract include ulceration, chronic rhinitis and pharyngitis, impaired lung function and emphysema (**Fowles** *et al.*, **2020**) .

6.2.3.4.Tin

Two studies extensively evaluated the sources of Sn in EC aerosol and strategies to reduce its levels by changing the design characteristics (**Wiliams ,2015**) . The authors suggested that Sn in EC was mainly emitted from the metal coating the thick Cu wires or the solder joints. It was trapped in the Poly-Fil fibers of the cartomizer. They revealed that both large-sized (> 500 nm) and nanometer-sized (< 0.1 mm) Sn and Ni particles were present in the aerosol. Besides, the coarse particles (> 1 mm) also included Ag, Fe, Al, and silicate and nanoparticles of Cr. Its concentration varied between and within the brands of EC. It was suggested that Sn could be reduced in aerosol by coating the thick wires with Ag and placing stable Sn solder joints outside the atomizing chamber. Further, joining the wires with brass clamps or brazing was more beneficial rather than soldering wires (**Lerner , 2015**). Changing the design was considered ideal to reduce leaching of Sn into the EC aerosol. The cartomizer fluid with Sn particles inhibited both attachment and proliferation of human pulmonary fibroblasts in a dose-dependent manner in this study. This shows that Sn in EC aerosols can affect the lung tissue (**Mikheev** *et al.*, **2016**).

Exposure to Sn(IV) oxide dust and fumes for more than 3 years results in benign pneumoconiosis called stannosis. Furthermore, animal studies have shown that Sn may cause anemia as indicated by decreased hematocrit, total erythrocyte, and hemoglobin levels. This was related to the negative influence of Sn on Fe and Cu metabolism. It reduced their levels in the blood, leading to anemia (**Rice** *et al.*, **2001**).

6.2.3.5.Copper

The Cu content in tobacco leaves was reported to be 156 mg/g. Serum concentrations in youngsters are about 0.95 mg/l, in adults 1.10 mg/l, and 1.31 mg/l in smokers (more than 10 cigarettes/day), although the differences did not reach significance. However, **Visser** *et al* **(2019)** described a significant correlation between plasma levels of Cu and lipid peroxidation in smokers.

Cu is essential at low concentrations. However, inhaled Cu is a respiratory irritant which causes alveolar migration of macrophages, eosinophilia, and formation of histiocytic and non-caseating granulomas. Furthermore, pulmonary fibrosis and fibrohyaline nodules may be formed. In animal studies, Cu strongly induced pulmonary inflammation than other transition metals (**Massadeh** *et al.*,**2010**). It is an active oxidation-reduction (redox) metal that has been determined at a significantly higher concentration in the blood of smokers (**Morsy and Khaled,2001**).

6.2.3.6.Lead

The Pb content of a cigarette is around 1.2 mg, and about 6% passes over to mainstream smoke, which is inhaled by smokers. **Mortada** *et al* **(2004)** report blood Pb levels in non-smokers of 101.6 + 30.9 mg/l and in smokers of 143.7 + 33.8. The dramatic difference between blood and serum Pb concentrations is due to the fact that Pb in the circulation is mainly bound to erythrocytes. Pb is eliminated from the body via urine, but this occurs slowly and accumulation in the skeleton is observed (**Jain ,2019**).

Other possible health consequences of lead accumulation are hypertension and peripheral arterial diseases, as well as cataract. As already stated, exposure to Pb may occur through inhalation of tobacco smoke including the EC aerosol. Pb is a major neurocognitive and kidney toxicant for children at a relatively low concentration (10 μg/dL) (Talio *et al.*, 2019). Children are at a high risk to accumulate the neurotoxic Pb in the brain and central nervous system, thus resulting in mental retardation and other neurological disorders. In addition, children from parents who smoke accumulate high levels of blood lead via passive smoking. These facts are just one further example that stresses the urgent need to protect children from active as well as passive smoking (Mason *et al.,* 2014).

It is present in both mainstream (exhaled by the smoker) and sidestream (from the burning cigarette) smoke, including the gas phase. A study reported that from 1988 to 1994, the US children exposed to second hand smoke had increased blood Pb levels. The latter was directly correlated to serum cotinine levels and the number of smokers at home. Furthermore, children living in urban areas or young adults of low socioeconomic status had high Pb exposure (Massadeh *et al.,* 2010). The second hand smoke exposure is an "unrecognized" source of relatively small particles of Pb which are more easily absorbed in the bronchial-alveolar region (Jelovcan *et al.*, 2003). In the present review, one study reported the presence of Pb in the open-wick ENDS devices while another study reported e-liquid to be the major source of Pb in EC aerosols (Exley *et al.,* 2006).

The various health effects of Pb in tobacco smoke are given in Table 3. Its effect on the nervous system has been explained here. The neurotoxic effects of Pb may range from alteration of nerve conduction velocity to encephalopathy. The symptoms may worsen to paralysis, convulsions, delirium, coma, or death (Palazzolo *et al.* ,2017).

The nervous system is affected through a combination of mechanisms, direct or indirect. It may directly alter the development of the system. This involves disruption of vital molecules during neuronal migration and differentiation or an interference with synapse formation (mediated by a reduction in neuronal sialic acid production) or a premature differentiation of glial cells. Besides, Pb may substitute for Ca and Zn in the central nervous system. This inappropriately triggers the processes dependent on calmodulin (Goniewicz *et al.*, 2014). It further interferes with the neurotransmitter release, energy metabolism (by activating protein kinase C), and Na^+/K^+ ATPase enzyme system in the cell membranes. Additionally, it accelerates the mitochondrial self-destruction and primes its apoptosis by inhibiting the Ca release (Allen *et al.,* 2016). The indirect effects result from interference with other body systems; for instance, Pb exposure causes hypertension and impairs the renal and thyroid function .A study in the present review reported that the design of ECs was a major source of Pb in aerosols. Therefore, it is imperative to incorporate Pb testing during chemical analyses of EC devices (Brookes *et al.*,2004).

6.2.3.7.Manganese

It is proposed that the Mn oxides oxidize Cr(III) to Cr(VI) and potentiate Cr toxicity (Schober *et al.*, 2014). Airborne Mn passes the closely regulated homeostatic control mechanism through the hepatic portal system. Mn concentrations in tobacco range from $155 - 400$ mg/g. The serum concentration ranges between 0.19 and 5.69 mg/l, but no relation to socioeconomic status, tobacco or alcohol consumption has been found. Compounds of Mn may induce or exacerbate asthma. Besides, Mn(II) has been associated with pulmonary inflammation in rats (Cooper *et al.,* 2016). Like Cu, further research is required to verify the adverse health effects of Mn in EC aerosol. Moreover, High concentrations of Manganese cause psychiatric syndromes (hallucinations, emotional lability), first described in 1800 as Manganese Madness. Today it is known as Parkinson-like syndrome (Hess *et al.*, 2017).

6.2.3.8.Mercury

Smoking plays a minor role as a source for Hg uptake by humans , only $5 - 7$ ng of mercury per cigarette are transferred into the smoke, and a number of studies failed to describe a significant correlation between the smoking status and blood or serum Hg levels. However, in one study smoking-mediated Hg delivery was reported to increase the number of sister chromatid exchanges (Jain *et al.*, 2019).

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6.2.3.9.Nickel

As for other metals in cigarette smoke, the question about Ni is whether the amount of Ni transferred from the smoke to the lung and then to the circulation is high enough to cause disease. The amount of Ni in the tobacco plant lies between 0.64 and 1.15 mg/g, and varies highly in cigarettes between 0.078 mg and 5 mg **(Tayyarah and Long ,2014)**.

Chronic long-term inhalation of Ni from EC aerosol may damage the lungs, liver, and kidneys in a manner similar to drinking water contaminated with Ni or inhalation as seen in refinery workers. The inhalation of Ni causes chronic active inflammation in the lungs, leading to alveolar epithelium hyperplasia, fibrosis, bronchiolization, alveolar proteinosis, and atrophy of the nasal olfactory epithelium **(Das et al.,2008)**. Furthermore, it causes significant oxidative stress in the lung tissue. This was revealed by increased levels of lipid peroxides with simultaneous reduction of superoxide dismutase, catalase, and glutathione peroxidase enzymes in the lungs **(Chun et al., 2017)**.

Many studies have suggested a close relationship between Ni exposure and lung and sino-nasal cancer. It was suggested that in workers exposed to Ni at concentrations of ≥ 15 mg/m^3, the risk of sinonasal cancer was higher than that of lung cancer. The risk was multiplied in workers who were heavy smokers **(Binazzi et al.,2008)**.

The Ni toxicity may affect the genes, blood, and nervous and reproductive systems. The genetic abnormalities are mainly related to DNA damage by binding to DNA and nuclear proteins. It is immunotoxic as it suppresses natural killer cells and interferon production. Besides, Ni ion may produce systemic allergic reactions by oxidizing to a low molecular weight protein called hapten. A study reported a significant increase in the levels of immunoglobulin (Ig)G, IgA, and IgM after Ni exposure **(Apostolou et al.,2012)**. Further, increased levels of other serum proteins, involved in cell-mediated immunity like α1-antitrypsin, α2-macroglobulin, and ceruloplasmin, have also been reported. Both Ni and Cr depress the circulating antibody response to viral antigens. Neurological signs like lethargy, ataxia, and reproductive toxicity, leading to spontaneous abortions and testicular degeneration, have also been reported **(Kienhuis et al., 2015)**.

6.2.3.10.Vanadium

The cigarette V concentration ranges between 0.49 – 5.33 mg/g (average: 1.11 mg/cigarette). About 60% of the vanadium remains in the ash, 8.3% in the cigarette filter and 31.3% in the smoke. In contrast to the gastrointestinal tract, the lungs absorb soluble Vanadium well. The excretion of the metal by the kidney is rapid (half life 20 – 40 h). V has been shown to have insulin-like properties in both animal and human studies **(Jain , 2019)**. The serum concentration of non-V exposed non smokers is 0.8 mg/l, but the effects of smoking on serum V levels have to our knowledge not been studied. Vanadium is known to provoke local irritation of the eyes and the upper respiratory tract (like rhinitis, cough, conjunctivitis, chest pain). It is also suspected to cause neurobehavioral impairments, which affect attention and visuospatial abilities **(Kuye Adesegun et al.,2021)**.

6.2.3.11.Zinc

Although zinc is present in cigarettes (average 24 mg/g) and about 70% is transferred to the smoke, Zn serum concentrations in the average population have not been found to be affected by the smoking status. However, there is evidence that under special circumstances, like pregnancy, mononuclear cells and maybe also other cell types have a reduced Zn content **(Miler and Hajek,2018)**. Reduced Zn levels have been shown to constitute a pro-carcinogenic factor as well as affecting immune system function. The mechanism by which cigarette smoking reduces Zn levels is thought to result from an increased blood Cd concentration, which induces the expression of metallo-thioneins, which bind Cd but also Zn **(Zhao et al., 2019)**.

6.3.Flavoring agents

Most flavoring agents are considered safe for the use in food products, however, the potential toxicity of the degradation products resulting from high-temperature evaporation is unknown. Findings from a study by **Lerner et al (2015)** showed that ROS production was significantly high in the mint flavor (0 mg nicotine) and classic tobacco flavor (16 mg nicotine) e-cigarette. Besides, **Zhao et al (2018)** found that classic tobacco-flavored e-cigarette reduced

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the production of ROS in the presence of nicotine. Flavoring agents can also cause cytotoxicity. For example, in a study by **Herrington** *et al* **(2017)** chocolate-flavored 2,5-dimethylpyrazine and vanillin were revealed to cause changes in cell physiology.

At sub-cytotoxic levels, exposure to 2,5-dimethylpyrazine for 24 h impeded the responsiveness of airway epithelial cells to signal agonists, which is important for water and salt balance at the airway surface. Furthermore, 2,5-dimethylpyrazine enhanced ionic conductivity, which is associated with the regulator ion channel through protein kinase A-dependent activation of cystic fibrosis transmembrane. Similarly, **Leigh** *et al* **(2016)**, revealed that coffee, menthol, and strawberry-flavored e-liquids reduced metabolic activity and cell viability, whereas coffee and strawberry-flavored e-liquids induced the production of cytokine in human bronchial cells.

6.4.Propylene glycol and vegetable glycerin mixture

Propylene glycol P.V and vegetable glycerin V.G. are carriers of nicotine and flavoring agents, they form the main components of e-liquids. In a study by **Lerner** *et al* **(2015)** the levels of ROS and oxidants detected in the air alone were significantly lower than in aerosols from P.G. and V.G. Additionally, findings from a study by **Scheffler** *et al* **(2015)** showed that cells exposed to P.G. or V.G. exhibited higher levels of oxidative stress than the control group. Moreover, the P.G./V.G. mixture reduced the cell survival rate of human airway smooth muscle cells, human adenocarcinoma alveolar basal epithelial cells, and embryonic kidney 293 cells in a dose dependent manner. A correlation between the P.G./V.G. ratio and the toxic components in e-cigarette vapor has been reported, for example, an experiment by **Leigh** *et al* **(2016)** exposed human bronchial epithelial cells to e-cigarette vapor and changed the basic components of e-cigarettes. Results showed that the metabolic activity of the human bronchial epithelial cells was reduced upon exposure to vapor from 50P.G./50V.G. and V.G., while in cells exposed to P.G., the release of cytokines was increased.

6.5.Other chemicals

Numerous components released as a result of the evaporation of e-cigarettes have been identified as potential carcinogens, they include aldehydes and metals (such as nickel, copper, and titanium) **(Hess** *et al.*, **2017)**. Studies have revealed that a quarter of e-liquids, including benzaldehyde, vanillin, and other flavoring agents contain aldehydes **(Tierney** *et al.*, **2016)**, and a large amount of formaldehyde, acetaldehyde and propionaldehyde are generated when heated to 150 °C **(Hutzler** *et al.*, **2014)**.

Aldehydes are the main irritants of the respiratory tract mucosa. Benzaldehyde, as the main ingredient in fruit flavorings, may irritate the respiratory tract and eyes. Exposure to acetaldehyde will affect the removal of mucus from mucosa or submucosa, leading to a decline in the host's defense capabilities. In a previous study, cherry-flavored e-cigarettes contained the highest concentration of benzaldehyde, and the dose of 30-puff benzaldehyde smoked from e-cigarettes was always higher than the dose inhaled from traditional cigarettes **(Kosmider** *et al.*, **2016)**. In addition, the thermal decomposition of solvents in e-cigarettes produced a series of organic compounds such as acrolein (extracted from P.G./V.G mixture). Notably, acrolein has been shown to induce chronic obstructive pulmonary disease COPD **(Moretto** *et al.*, **2012)**. Another previous investigation indicated that copper metal nanoparticles could reduce the mitochondrial membrane potential and stimulate the production of mitochondrial ROS in human lung fibroblasts **(Lerner** *et al.*, **2015)**. Tobacco-specific nitrosamines, which are carcinogenic compounds in traditional cigarettes, have been detected in the vapor **(Goniewicz** *et al.*, **2014)** and liquid of e-cigarette, even at a lower content than in traditional cigarettes.

7.Toxicity mechanisms of E-cigarettes

The toxic effects of E-cigarettes are related to oxidative stress, inflammation response, and DNA damage. The toxic outcome of electronic cigarettes is shown in Figure (4)

7.1.Oxidative stress

Oxidative stress is defined as "An imbalance between oxidants and antioxidants in favor of the oxidants, disrupting redox signaling and control and/or cause molecular damage" **(Sies, 2015)**. In other words, when the level

of reactive oxygen species is higher than the activity of antioxidant enzymes, oxidative stress occurs (**Burton and Jauniaux, 2011**). Reactive oxygen species (ROS) can cause membrane lipid rupture, protein denaturation, DNA damage, mitochondrial dysfunction and other cellular macromolecular damage, thus severely altering signal transduction and cell metabolism. Following existing reports, exposure of human lung epithelial, bronchial epithelial cells (**Scheffler** *et al.*, **2015**), human pulmonary vascular endothelial cells (**Putzhammer** *et al.*, **2016**) and human umbilical vein endothelial cells to E-cigarettes can generate oxidative stress, increase endothelial cell permeability and cause inflammation and cytotoxicity (**Schweitzer** *et al.*, **2015**).
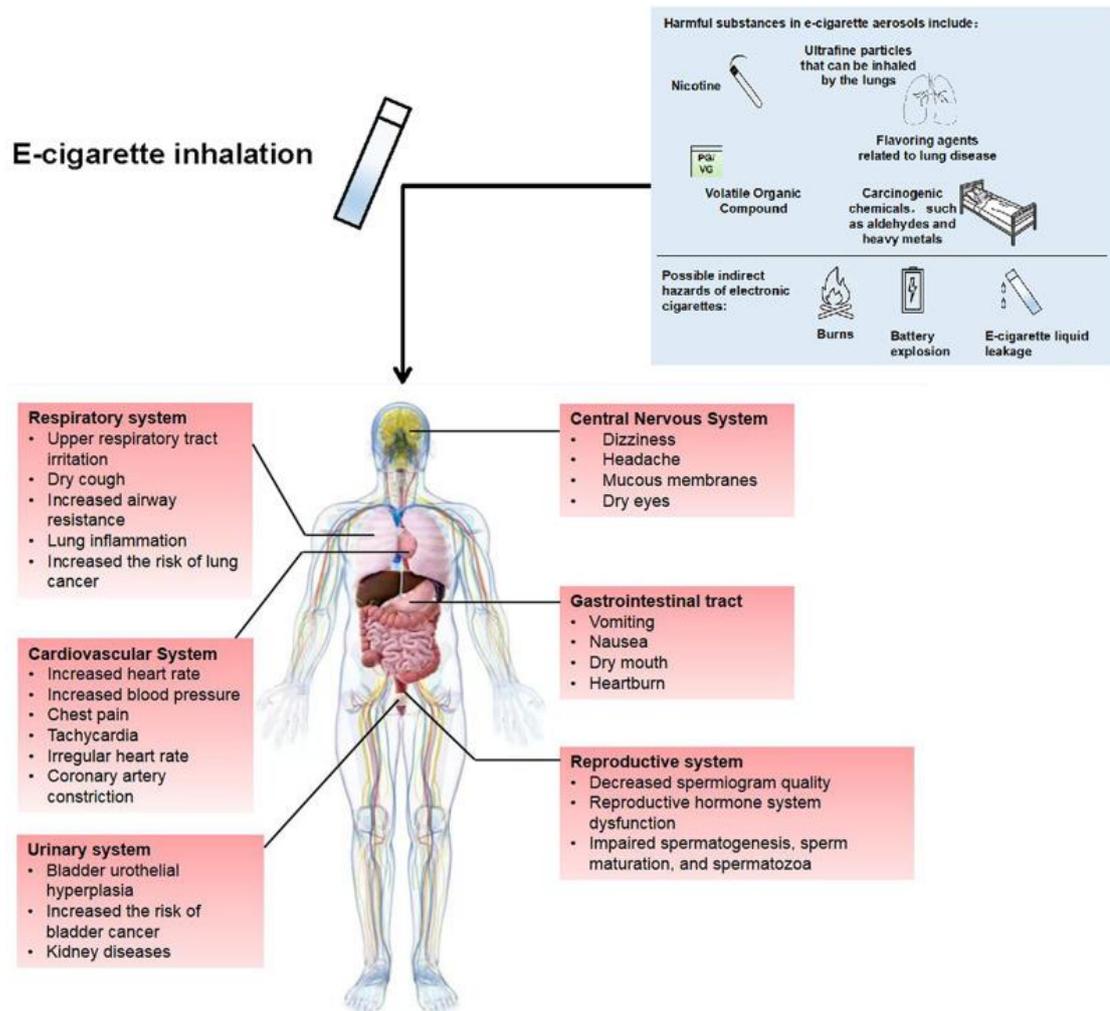

Figure (4) Toxic and health outcomes of electronic cigarettes. E-cigarettes are detrimental to the central nervous system, respiratory system, cardiovascular system, urinary system and reproductive system and the specific manifestations (**Lerner** *et al.*, **2015**).

7.2. Inflammation response

Inflammation is an adaptive response triggered by harmful conditions such as physical damage, chemical damage and tissue damage caused by certain antigens and antibodies, etc. According to reports, E-cigarettes induce inflammation response such as the experiments by **Iskandar** *et al.* **(2016)** and **Antherieu** *et al.* **(2017)**, who exposed NHBE cells and BEAS-2B cells to e-cigarette aerosols, revealed an elevated release of some proinflammatory mediators. Similarly, a study by Lerner et al. (2015) proved that the flavoring by cinnamaldehyde stimulated human lung fibroblasts to generate large amounts of inflammatory cytokine IL-8, the normal human bronchial epithelial (NHBE)

cells cultured at the air-liquid interface to E-cigarettes containing nicotine and found that the secretion of IL-6 and IL-8 increased after exposure to nicotine for 5 days.

7.3.DNA damage

DNA damage may originate from external physical radiation, chemical substances, endogenous reactive oxygen species, or metabolic byproducts, potentially causing cancer. Continuous DNA damage drives the aging process **(Ou and Schumacher, 2018)**. Electronic cigarettes can also cause DNA damage, for example, a study by Lerner et al. showed that copper nanoparticles and e-cigarette aerosols induced mitochondrial ROS production would cause DNA fragmentation in lung fibroblasts **(Lerner *et al.,* 2016)**. Similarly, a study of **Anderson *et al.*(2016)** revealed that e-cigarette aerosol induced ROS, caused DNA damage, and significantly reduced cell viability in a concentration-dependent manner. Moreover, another study by **Lee *et al* (2018)** found that nicotine and its metabolite, nicotine-derived nitrosamine ketone, can be further transformed into aldehydes and in lung, bladder, and heart tissues of humans and mice. The nicotine and nicotine-derived nitrosamine ketone induce DNA damage to enhance mutational susceptibility and induced tumorigenic transformation of human lung and bladder epithelial cells.The above reports confirm that DNA damage caused by E-cigarettes can not only lead to lung toxicity, but also cause toxicity to other organs such as the bladder.

8.Effects of E-cigarette on health

8.1.Effects of E-cigarette use on the pulmonary system

Cigarette smoking is the major cause of lung cancer and chronic obstructive pulmonary disease COPD.COPD, accounting for about 90% of all cases (38–40). The smoke contains numerous toxicants that promote inflammatory responses that contribute to the risk for these diseases. Inflammation is considered a hallmark of cancer and COPD. The proinflammatory effects on the lung are observable in healthy smokers before the onset of disease **(Joshi *et al.,* 2018)**.

It is important to study multiple components within e-cigarette aerosols, because while nicotine is a cholinergic drug and may have effects that directly mirror those seen in conventional tobacco use, tetrahydrocannabinol THC activates different molecular pathways, and will have different effects on airway epithelium, smooth muscle cells and overall lung function. Chemicals within e-cigarette aerosols first meet the airways, prior to absorption into the bloodstream where they come in contact with vascular endothelial cells while circulating to reach the other organ systems, including the heart. Thus, a great many of the research studies published to date have focused on the direct effects of e-cigarette aerosols on the pulmonary system **(Tierney *et al.,* 2016)**.

In addition to understanding the effects of e-cig aerosols on the conducting airways, it is important to understand how these aerosols affect the alveolar epithelium, which constitutes most of surface area in the lung and is subject to high levels of particle deposition. Injury to the alveolar-capillary barrier can produce severe physiological consequences, including acute respiratory distress syndrome ARDS. Notably, cigarette smoke exposure increases the risk of developing ARDS in a variety of settings, including following blunt trauma and in non-pulmonary sepsis **(Madison *et al.,*2019)**.

Similarly, healthy smoking volunteers compared with nonsmokers manifest greater alveolar-capillary barrier permeability to protein after inhaling nebulized lipopolysaccharide. Cigarette smoke activates alveolar macrophages and airway epithelial cells to release proinflammatory cytokines, resulting in the recruitment of infiltrating inflammatory cells from the blood to the lung. At the same time, normal protective mechanisms for adequate tissue repair by fibroblasts are hindered by cigarette smoke: proinflammatory pathways are upregulated and anti-inflammatory ones are downregulated **(Miyashita *et al.,* 2018)**.

There are numerous studies indicating that induced sputum has higher inflammatory cell content (e.g., neutrophils) in smokers compared with nonsmokers; counts tend to be increased with increased smoking exposure. Sputum neutrophils decreased after 6 weeks of smoking cessation in two studies; in a small sputum study, there was not a change 4 weeks after quitting **(Shields *et al.,* 2017)**. Macrophages decrease as early as 1 week following smoking cessation. On the basis of bronchoscopy data, total cell counts, macrophages, lymphocytes, neutrophils, eosinophils

and basophils, are much higher in smokers compared with nonsmokers. Important evidence comes from smoking cessation studies (**Staudt *et al.,* 2018**). In a study of 28 smokers who underwent bronchoscopy, 12 months after quitting they had reduced numbers of inflammatory cells compared with those who continued smoking (**Pisinger and Dossing,2014**).
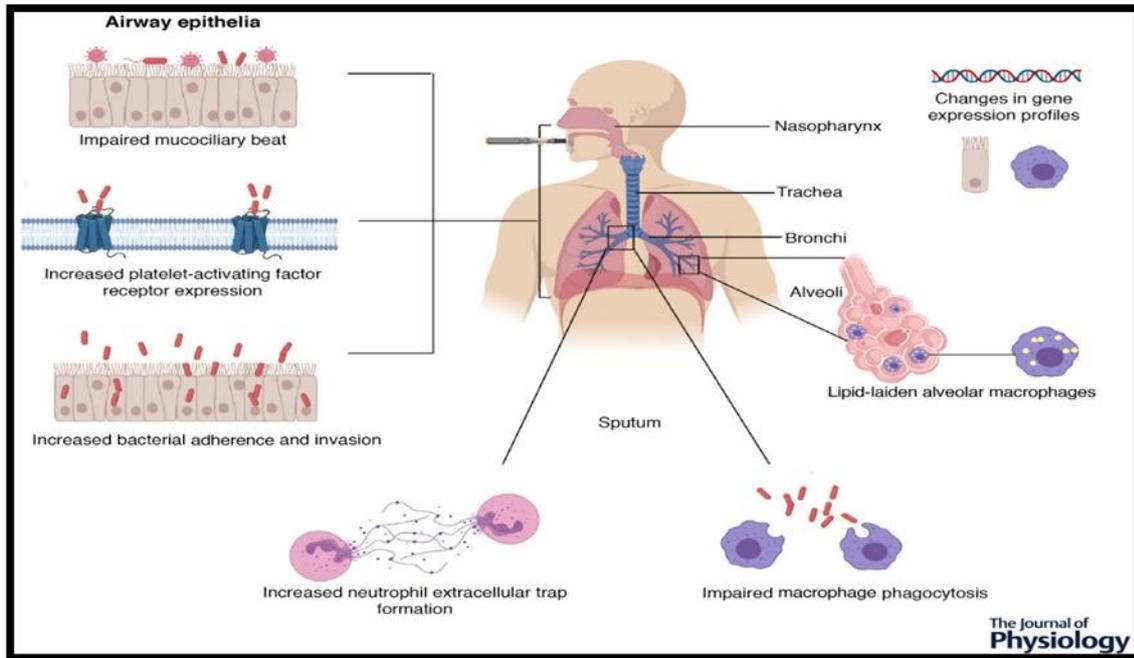

Figure (5):The effect of E-cigarette exposure on the pulmonary immune system and host defence (**Pisinger and Dossing,2014**).
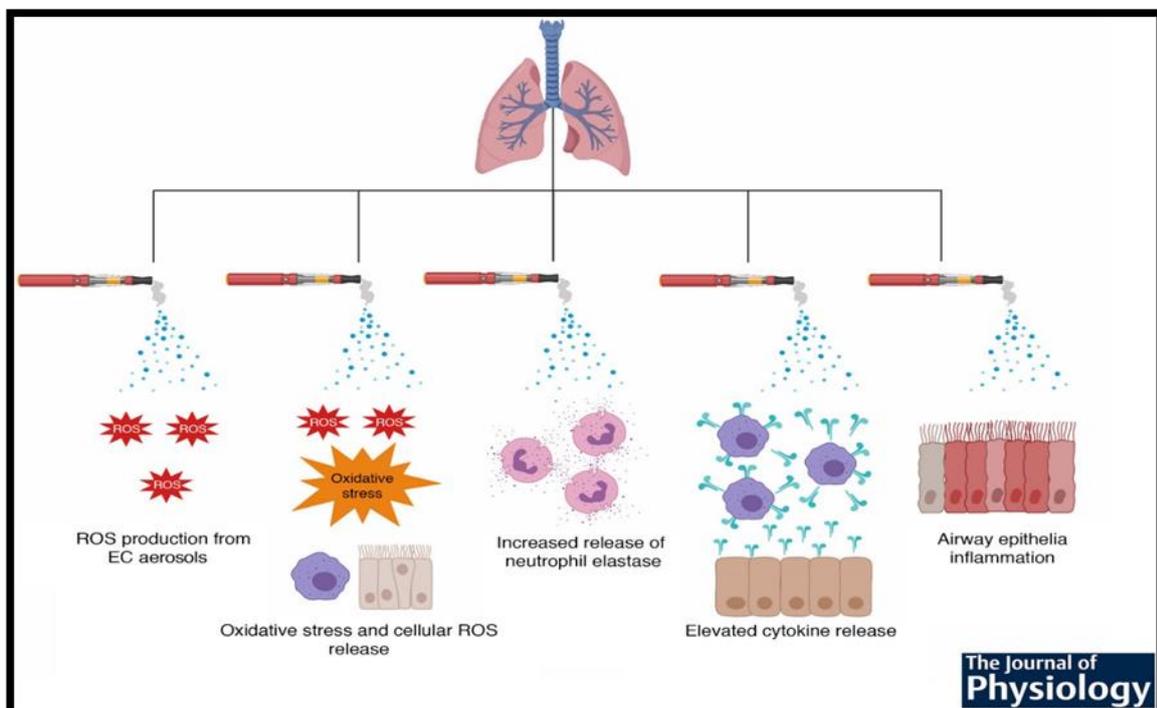

Figure (6) The pro-inflammatory effects of E-cigarettes(**Pisinger and Dossing,2014**).

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Airway inflammation and oxidative stress are key events in chronic conditions such as asthma, COPD and infections such as pneumonia, and can be highly deleterious to the pulmonary system. These processes all have a similar degree of inflammation from the same pool of pro-inflammatory cytokines. Key studies include a murine model of chronic EC exposure, in which mice exposed to EC vapour for 1 hour daily over the course of 4 months subsequently displayed some features akin to human COPD, including airway hyper-reactivity, lung tissue destruction and increased expression of pro-inflammatory cytokines in lung homogenates, such as interleukin (IL)-8 **(Garcia-Arcos et al., 2016).**

The cells driving inflammation after EC exposure include Alveolar macrophages AMs, which are observed to secrete elevated levels of pro-inflammatory cytokines and chemokines (such as IL-6, tumour necrosis factor α and IL-8) following EC extract exposure in vitro **(Scott et al., 2018).** Another neutrophil-derived mediator that has a major role in pulmonary host defence, is neutrophil elastase, which if unchecked has the potential to cause considerable pulmonary damage and induce the release of pro-inflammatory cytokines. It is therefore of interest, that EC-exposed neutrophils in vitro release increased elastase levels compared to unexposed controls **(Higham et al., 2016).** Reactive oxygen species ROS production is part of normal cellular metabolism, participating in maintenance of cellular redox homeostasis under physiological conditions. Excessive ROS production, however, elevates oxidative stress and airway inflammation. EC vapour is reported to contain up to 10×10^3 free radicals per puff ,with high oxidative potential **(Miyashita et al., 2018).**

8.2. Effects of E-cigarette Aerosols on Cardiovascular Health

E-cigarette smoking every day doubles the risk of heart attack according to a study conducted by the Center for Tobacco Research and Educational Center at University of California San Francisco (UCSF) in 2018 **(Sapru et al., 2020).** The study provides the first evidence that electronic nicotine devices have a significant impact on human health. The new study of nearly 70,000 people found that e-cigarette users' increased risk of heart attack seems to be one of the main toxic effects of E-cigarettes. Overall, people who use traditional cigarettes and E-cigarettes daily increased the risk of heart disease by five times. Nicotine is the primary agent in both regular cigarettes and E-cigarettes, and it is highly addictive. It is well-documented that nicotine is present in electronic cigarettes. The sympathomimetic effect of nicotine may increase myocardial contractility and heart rate, reduce insulin sensitivity, and increase coronary resistance, consequently increasing the cardiovascular risk of smokers to a certain extent . Thermal degradation of propylene glycol and vegetable glycerin in E-cigarettes can generate carbonyl compounds such as formaldehyde, acetaldehyde, and acrolein, which may cause cardiomyopathy **(Henning et al., 2017).** Various flavors used in E-cigarettes have potential harm to the human body. For example, cinnamaldehyde may exert cardiotoxic effects, heavy metals such as lead and cadmium have also been detected in some e-cigarette aerosols and potentially causes carcinogenicity **(Hess et al., 2017).** The above reports confirm that electronic cigarettes can damage the cardiovascular system.

There is an urgent need to determine both the acute and the long-term effects of E-cigarettes on the hearts and blood vessels of healthy adults and children, as well as those with either risk factors for cardiovascular disease or outright cardiovascular disease and to determine the comparative safety of E-cigarettes relative to combustible cigarettes **(Bhatnagar et al., 2014).** Multiple studies have been conducted on the acute effects of e-cigarette use on cardiac physiology, including heart rate (HR) and blood pressure (BP). Smokers who were given E-cigarettes with at least 18 mg ml–1 of nicotine were noted to have increased HR in four separate acute e-cigarette exposure studies **(Vlachopoulos et al., 2016; Chaumont et al., 2018).**

In addition, epidemiological studies have also proved that E-cigarettes have detrimental impacts on the human cardiovascular system. For instance, **Kerr et al (2019)** evaluated the changes in blood vessels after short-term inhalation of vapor from an e-cigarette and measured endothelial progenitor cells (EPC) and microvesicles (MVs) in healthy young volunteers. Moreover, they classified 16 healthy seldom smokers into two groups (with or without exposure to 10 puffs of e-cigarette vapor for 10 min), in a crossover design. It was found that inhalation of 10 puffs of

vapor from e-cigarette increased the EPC in healthy volunteers. As previously demonstrated, this increase is similar to that of smoking a conventional cigarette (**Antoniewicz et al., 2016**).

Also, **Carnevale et al.** compared the effects of E-cigarettes and traditional cigarettes on endothelial function and oxidative stress in non-smokers and healthy smokers. They conducted a single-blind crossover study in 40 healthy participants. Results revealed that smoking has a significant effect on all markers for flow-mediated dilatation (FMD) and oxidative stress. However, there is no statistical difference in the biological effects of vitamin E levels and FMD between electronic cigarettes and traditional cigarettes (**Carnevale et al., 2016**).

As mentioned earlier, propylene glycol and vegetable glycerin in E-cigarettes may be carcinogenic when heated at high temperatures. Besides, **Chaumont et al**(2018) evaluated the different effects of propylene glycol and vegetable glycerin mixture and nicotine on arterial stiffness, microcirculatory function, oxidative stress, and hemodynamic parameters in a single blind, randomized, three-period crossover design study. They found that in young, healthy smokers, nicotine-free propylene glycol and vegetable glycerin mixtures (50:50) were acutely exposed to high temperature evaporation which did not alter microcirculatory function, arterial stiffness, and oxidative stress. Atomizing the same mixture with nicotine could increase arterial stiffness, reduce microcirculation endothelium-dependent functions, cause a continuous rise in blood pressure and heart rate, and increase plasma myeloperoxidase level (**Chaumont et al., 2018**).

8.3. Effects of E-cigarette heavy metals on oral health

The oral tissues most likely to be affected are periodontal ligament and oral epithelium. The role of E-cigarette smoke in promoting chronic periodontitis and oral cancer is well established. However, there is negligible evidence on the role of ECs on oral tissues. A study demonstrated that ECs with flavorings increased the oxidative/carbonyl stress and inflammatory cytokine release in human periodontal ligament fibroblasts, human gingival epithelium progenitors pooled (HGEPp), and epigingival 3D epithelium. Furthermore, increased levels of prostaglandin E2 and cycloxygenase-2 were demonstrated. They upregulated the receptor for advanced glycation end products (RAGEs) through EC-mediated carbonyl stress in the gingival epithelium. They even increased DNA damage along with histone deacetylase 2 (HDAC2) reduction via RAGE-dependent mechanisms in the gingival epithelium. This suggests that increased oxidative stress, pro-inflammatory, and pro-senescence responses are induced by ECs that deregulate repair in periodontal ligament cells. Further, the menthol derivatives in e-liquids reduced the fibroblastic proliferation (**Rouabhia et al., 2017**).

Another study investigating the effects of EC vapor on human gingival epithelial cells demonstrated altered morphology of cells from small cuboidal to large undefined shapes. Both single and multiple exposures led to bulky morphology with large faint nuclei and enlarged cytoplasm. It also increased L-lactate dehydrogenase (LDH) activity in the targeted cells, specifically with repeated exposures. The percentage of apoptotic/necrotic epithelial cells was also increased. This was attributed to over activity of the caspase-3 pathway. These morphological and functional changes in gingival cells could enhance progression of oral cancer (**Arora et al., 2009**).

Specific metals like Ni, Pb, and Cr are more concentrated in EC aerosol than in burnt tobacco .Therefore, they are highly likely to influence the gingival epithelium, periodontal ligament, and oral mucosa. Unfortunately, this area has not been explored. However, their adverse effects on oral tissues when released from the CCs have been widely demonstrated. For instance, a study reported that cigarette smoke was a major source of Cd (**Wallin et al., 2016**). Chronic low levels of Cd were linked to decreased bone mineral density and osteoporosis .

It was suggested that Cd increased the serum concentration of cross-linked telopeptide of type 1 collagen (a marker of bone resorption) and decreased the activity of serum alkaline phosphatase (a marker of bone formation). This disturbed the normal bone metabolism and enhanced the levels of inflammatory mediators in osteoblasts. It even caused Ca release from cells at very low concentrations. Therefore, Cd might disturb alveolar bone remodeling in periodontal disease, favoring the resorption (**Khlifi et al., 2013**).

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Another study compared toxic heavy metal accumulation in the supragingival dental calculus of smokers and non-smokers. It was found that the levels of metals like As, Cd, Pb, Mn, and vanadium were significantly higher in the dental calculus of smokers than non-smokers. Their overexpression was significantly correlated to chronic tobacco smoke exposure. Similarly, accumulation of Cd and Pb has been reported in the teeth of smokers (**Won** *et al.***, 2013**). As already stated, these metals are carcinogenic and may trigger oral cancer by impairing pathways for the anti-oxidative metabolism, Ca-dependent apoptotic cell death, stimulating free radical production, and inducing oxidative stress.

Another study evaluated the concentrations of As, Cd, Cr, and Ni in healthy and tumor tissues of patients with head and neck cancer. Their levels were about 1.3 to 3.4 times higher in tumor tissues than in healthy tissues. Besides, Ni has been associated with oral cancer . Although this association was seen with Ni present in soil, water, and air, the linkage between Ni from inhaled EC aerosol and oral cancer is yet to be investigated (**Su** *et al.***, 2010**).

8.4. Effects of E-cigarette on Platelets

Both mainstream and side stream cigarette smoke have been shown to cause heightened platelet activation, adhesion aggregation and inflammation (**Kashiwagi** *et al.***, 2017**). Based on the possible clinical implications of altered platelet exposure, the potential effects of E-cigarette extracts on platelet function have also been investigated. Platelet activation and aggregation were increased following exposure to e-liquid aerosol extracts. Platelet adhesion potential for fibrinogen and von Willebrand factor were also increased, indicating that e-cigarette extracts had a pro-thrombotic effect. In this study, the effects were caused by the non-nicotine constituents of E-cigarettes, while other studies have shown increased platelet aggregation and/or activation after exposure to e-cigarette vapor with nicotine (**Qasim** *et al.***, 2018; Ramirez** *et al.***, 2020**).

8.5. Effects of E-cigarette Aerosols on Liver, Kidney and reproductive Health

In addition to the toxicity to the main contact area (respiratory system), and cardiovascular system, electronic cigarettes cause toxicity to other human systems such as the, reproductive system, liver and nervous system.

Experiments have shown that E-cigarettes have certain toxicity to the reproductive system and liver. For instance, **Golli** *et al* (2016) in their experiment, administered an intraperitoneal injection of e-liquid into adult rats once a day for 4 weeks, they found that E-cigarettes had a damaging effect on the kidneys . Further, they explored the effects of e-liquid on the liver and testes of rats through intraperitoneal injection for 4 weeks. Notably, they revealed that in the liver, e-liquids potentially promoted oxidized tissue damage, leading to visible histopathological changes. Nicotine alone caused less oxidative stress and histopathological disease, whereas e-liquids used with nicotine resulted in more histopathological damage. Thus, it was suggested that the e-liquid alone can cause liver toxicity, which can worsen upon nicotine supplementation (**El Golli** *et al.***, 2016**).

Furthermore, e-liquids without nicotine have been shown to induce toxicity in rat testis by disrupting oxidative balance and steroidogenesis, similar to nicotine-containing e-liquids (**El Golli** *et al.***, 2016**). A study by **Kennedy** *et al* (2017) assessed the effect of E-cigarettes on developing embryos, by exploring the effects of several atomized E-cigarette liquids in the *in vivo* craniofacial model. Results showed that exposure to E-cigarette aerosol during embryonic development could induce midface hypoplasia and median facial clefts (**Kennedy** *et al.***, 2017**). Also, **Lauterstein** *et al.* investigated the effects of E-cigarettes on embryo development, using pregnant mice and received E-cigarette aerosol during pregnancy and lactation, 3 h/day and 5 days/week. They reported that E-cigarette aerosols induced sex-dependent gene expression changes with or without nicotine, which was related to expected adverse neurobiological and neurobehavioral results, similar to those associated with early exposure to traditional cigarette (**Lauterstein** *et al.***, 2016**). In addition, **Zelikoff** *et al* (2018) used the same experimental method to study the effects of e-cigarette aerosols on the activation of astrocytes and microglia in the developing brain. Results demonstrated that e-cigarette constituents other than nicotine, for example, flavoring agents, could cause biological damage in the developing central nervous system .

Furthermore, **Prasedya** *et al*(2020) exposed BALB/c mice to air, traditional cigarettes and E-cigarettes for 14 days. Then, they evaluated the cognitive function of the mice by using a maze test. It was found that mice exposed

to traditional cigarettes and E-cigarettes delayed the time to find food. This means that traditional cigarettes and E-cigarettes exposure may cause damage to the olfactory system. And it is worth noting that E-cigarettes exposure may cause abnormal memory function in mice. Histological staining of the cerebral cortex of mice in the E-cigarettes exposure group showed inflammatory reactions, such as necrosis and cytoplasmic vacuolation. Immunohistochemical staining showed that the pro-inflammatory cytokine TNF-α was highly expressed in the group exposed to E-cigarettes and traditional cigarettes. Therefore, E-cigarettes have similar toxicity characteristics as traditional cigarettes, which may have negative effects on brain function **(Prasedya *et al.,* 2020).** There are a few experiments to study the harm of E-cigarettes to other tissues and organs, and more data are needed to draw conclusions. In short, these findings are expected not only to raise awareness of the negative health effects of the growing popularity of E-cigarettes, but also increase information to consider the safety of E-cigarettes.

9.Analysis and determination of heavy metals in E-cigarettes

Heavy metals analysis to electronic cigarette refill liquids is usually done employing Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) **(Bansal, 2016).** **Palazzolo *et al.,*(2017)** utilized ICP-MS to quantify metals in e-liquids in their package. **Hess *et al* (2017)** analyzed 10 different brands of refill liquids using ICP-MS and noted remarkable variability in concentrations within and between brands. Wide ranges in the levels of metals are also reported by **Cheng (2014).** ICP spectrometry is ideal for a rapid and sensitive multi-element analysis as it is characterized by good quantitative capability, limited spectral and chemical interferences. Detection limits for most elements are in the ppb range or less. ICP-MS was used in all cases. Despite all the advantages, its high price, the operational cost, the need for expert personnel and large quantities of samples for the analysis, appear as major drawbacks. Total Reflection X-Ray Fluorescence spectrometry (TXRF) was employed for the elemental analysis of various refill liquids. This technique exhibits several advantageous features, which include simultaneous multi-element analysis, low matrix effects, relatively short analysis time (100s-1000s), ease of operation, simple sample preparation, analysis of small sample amounts (µg or µL range) and low detection limits reaching the ppb level **(Antosz *et al.,* 2012).** This technique is applicable to various types of specimens and quantification is less complicated using an internal standard.

In addition, mobile TXRF instruments are available offering the possibility of on-site analysis. The physical main principle of TXRF analysis is exactly the same as for the conventional energy dispersive X-ray Fluorescence analysis. However, there are important differences with regard to the excitation and detection geometry as well as the sample preparation. The sample is analyzed as a thin layer on a flat, highly polished sample carrier. The excitation beam is adjusted to the sample carrier surface with a grazing incident angle. This means that just very little scattering radiation of the sample carrier is detected in the measurement spectrum. The detector is placed very close to the sample carrier and detects the X-ray fluorescence radiation of the sample with very high efficiency. This setup gives rise to very efficient radiation detection, increased sensitivity as no secondary excitation effects take place and simpler quantification process by means of internal standardization. TXRF has been applied for the elemental analysis of various geological samples such as rock, mineral, ore and natural water **(Cherkashina *et al.,* 2014)**, for the determination of iodine in dietary supplement products, for elemental analysis of airborne particulate samples and for trace elements in apatitic rocks

9.Conclusion

In general, data from several studies conducted in rodents and model organisms show that E-cigarettes are harmful. There is a rapidly growing body of evidence derived from in vitro, animal, and human studies that e-cigarette use may have health risk for the public. Moreover, it suggests that the damage caused by electronic cigarettes to the respiratory system may be related to inflammation and oxidative stress. However, due to differences in exposure methods and species, extrapolation to the human body has great uncertainty.

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