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The Toxic and Pathophysiological Aspects of Smoking Related to the Most Usual Forms of Use and Exposure to Tobacco Smoke – A Systematic Review

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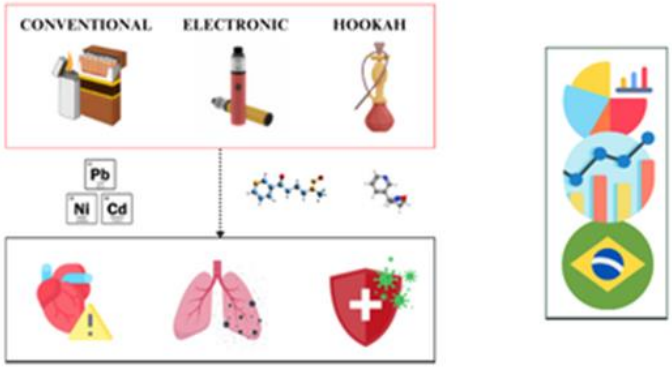
This review aimed to delineate the toxicity and pathogenicity of conventional, electronic, and hookah cigarettes concerning active and passive smoking, bringing epidemiological aspects related to Brazil. The research, conducted on the descriptors "nicotine extraction", "nicotine and toxicology" and "smoking and pathophysiology", in the Science Direct, Pubmed, Scopus, and Web of Science databases, resulted in the selection of 28 articles (2010-2022). Outside, also, exposed by the IBGE System of Automatic Recovery (SIDRA) in association with the National Health Survey (PNS), the percentages related to national smoking. It was found that the main affected systems are immunological, respiratory, and cardiovascular, with occurrences of cytotoxicity and genotoxicity, factors that are associated mainly with the presence of nicotine and toxic metabolites in cigarette smoke. It was also showed that levels of heavy metals can be found in e-cigarettes and that hookah. Conventional cigarettes still are the most harmful form of smoking. It is seen that indirect smoke stands for a serious public health problem because 80% of nitrosamines deposited on surfaces are not removed under normal ventilation conditions. It is concluded that smoking, regardless of the form of exposure, is harmful to the biological system, directly and indirectly affecting essential organs for human survival.

Graphical abstract



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1. Introduction

Currently, about 7 million deaths annually result from smoking. Nevertheless, the life expectancy of smokers is also reduced, with a decrease of about 20 years, compared to nonsmokers [1]. These data relate mainly to active smokers,

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to those who are passively exposed, that is, who inhale residual tobacco smoke, also suffer the consequences, since, for every eight smokers who die from smoking-related diseases, one does not suffer smoker dies from passive exposure [2, 3].

Smoking, whether active or passive, can cause the development and/or progression of diseases that affect the cardiovascular system [4], through hemodynamic and autonomic changes, oxidative stress, inflammatory process, thrombosis caused by pathological platelet aggregation [5], hyperlipidemia and endothelial dysfunction, the main factor associated with smoke-induced atherogenesis, with a consequent reduction in the bioavailability of nitric oxide, a potent endogenous vasodilator [1,6].

In addition, the respiratory system is affected by diseases that limit airflow and lead pulmonary endothelial cells to senescence, as occurs in Chronic Obstructive Pulmonary Disease (COPD), which manages 45% of deaths attributed to tobacco [7]. Recent studies show that nicotine causes overexpression of the angiotensin II-converter enzyme (ECA-2), one of the only recognized receptors of SARS-CoV-2 in the airways of smokers and patients with COPD, promoting, in cooperation with host proteases, the entry of the virus into human cells [8]. Thus, given the pulmonary pathological dysfunctions generated by smoking, as well as the action of nicotine concerning Covid-19 virus receptors, smoking behaves as a risk factor for viral invasion by a coronavirus.

Cigarette smoke is also harmful to the immune system because nicotine is an immunosuppressive agent that inhibits innate and adaptive immune responses [9]. Tobacco alkaloid exerts its effects through nicotinic acetylcholine receptors (nAChRs), however, the expression of these receptors in the epithelium of the airways is associated with the progression of neoplasms because of pro-angiogenic and anti-apoptotic actions of nicotine. This is one of the reasons why smoking is at the heart of carcinogenesis [10].

Unlike industrialized cigarettes, which work from the burning of tobacco, with the production of numerous toxic products from combustion, such as tar and carbon monoxide, electronic cigarettes (e-cigarettes), battery-operated devices, act by aerosolizing an e-liquid consisting of sub myotic droplets of glycols, such as propylene glycol ($C_3H_8O_2$), vegetable glycerin ($C_3H_8O_3$), flavorings, and nicotine ($C_{10}H_{14}N_2$) [11, 12]. Despite the modernization in nicotine delivery mode, heating e-liquids at elevated temperatures releases carcinogenic carbonyl compounds such as formaldehyde (CH_2O), acetaldehyde (C_2H_4O), and acrolein (C_3H_4O) [12, 4]. Other compounds such as nitrosamines, volatile organic compounds such as toluene ($C_6H_5CH_3$) and xylene (C_8H_{10}), phenolic compounds such as catechol ($C_6H_6O_2$) and hydroquinone ($C_6H_4(OH)^2$), and heavy metals such as lead (Pb), nickel (Ni), and copper (Cu) are also seen [13-15]. Thus, inhalation of these substances together can culminate in damage to the respiratory, circulatory, digestive, and nervous systems [16]. Thus, although the capitalist media industry portrays e-cigarettes as safety devices, including as a less toxic alternative to traditional smoking [17], the statements are controversial and lack adequate scientific evidence [12, 18].

In addition, the ultra-way of using nicotine is with hookahs, which have about 100 million users worldwide [19]. In Brazil, 0.4% of the population uses this form of smoking, with the highest prevalence being among young people aged 18-24 [21]. This form of smoking is based on the burning of coal that is placed above tobacco. The smoke generated passes through a hose with a nozzle and is therefore cooled. Although beliefs suggest that smoke toxicity is retained in water, studies indicate that tobacco used for hookah in a single session produces 100 times more tar, 4 times more nicotine, 11 times more carbon monoxide, and up to 5 times more polycyclic aromatic hydrocarbons than a single industrialized cigarette [20]. Despite the alarming data, hookah smoke ends up being neglected by tobacco control policies, thus resulting in impertinent data about its effect on human health [19].

Thus, it exposes the relevance and the need to produce recent studies aimed at the synthesis and review of the characteristics and consequences of active or passive exposure to the various forms of smoking present in contemporary times.

2. Material and Methods

This article consists of a literature review of the system type with descriptive character. For the preparation of the present study, primary scientific articles were used, published between 2010 and 2022, in English, Portuguese, and Spanish, which proved to have data relevant to the synthesis of the research. The inclusion criteria were based on experimental trials, with relevant evidence for the design and association of pathophysiological conditions resulting from smoking, whether active or passive, in the most usual forms of use, such as conventional cigarettes, e-cigarettes, and hookah. Articles with no full text available and outside the period and/or pre-established languages have been discarded, according to exclusion criteria. Finally, updated epidemiological data on the national percentage (Brazil) of users of industrialized cigarettes and tobacco products and individuals exposed to secondhand smoke, whether in workplaces or home environments, were analyzed and exposed in this review.

The articles were obtained through the Science Direct, Pubmed, Scopus, and Web of Science databases, through the search for the following descriptors: nicotine extraction, nicotine and toxicology and smoking and pathophysiology. The percentage data were obtained by the IBGE System of Automatic Recovery (SIDRA) in association with the National Health Survey (PNS). The search period was conducted between May and September 2022. The selected articles (28) were analyzed according to the descriptor, database, method, results, and conclusions. Figure 1 shows the layout of the pre-selection and selection process used.

3. Results and Discussion

3.1. Numerical Representations of Smoking in Brazil

According to the National Cancer Institute (INCA), all modes of tobacco consumption and its derivatives can cause severe damage to the health of smokers, whether they are active or passive. In 2014, the World Health Organization (WHO) said that there are no safe levels of exposure to smoke emitted by smokers and tobacco-burning products, a fact that includes conventional, electronic, and hookah cigarettes [21].

In Brazil, the prevalence of smoking is still significant. In 2019, during the National Health Survey (PNS), the Brazilian Institute of Statistics and Geography (IBGE) enabled a household health survey by probabilistic sampling, whose one of the aims was to produce data about the lifestyle of the Brazilian population living in private households, whether in urban or rural areas. The information collection technique was

based on a computer-assisted personal interview (CAPI).

Figure 2 shows the percentages of smoking in the Brazilian population in 2019. The percentage of smokers was 12.6% of the total national population, in addition, it was found that 9.9% consist of conventional cigarette users and 12.8% of users of tobacco products. The percentage of individuals exposed to secondhand smoke at home (9.2%) and at work (8.4%) was also figured out.

It is noted that the higher prevalence is related to the male sex, except when it comes to indirect smoking in the home environment. Although the use of e-cigarettes has increased exponentially in recent years, especially among 13- and 17-year-olds [21], there is a lack of statistical data on the prevalence of specific forms of smoking.

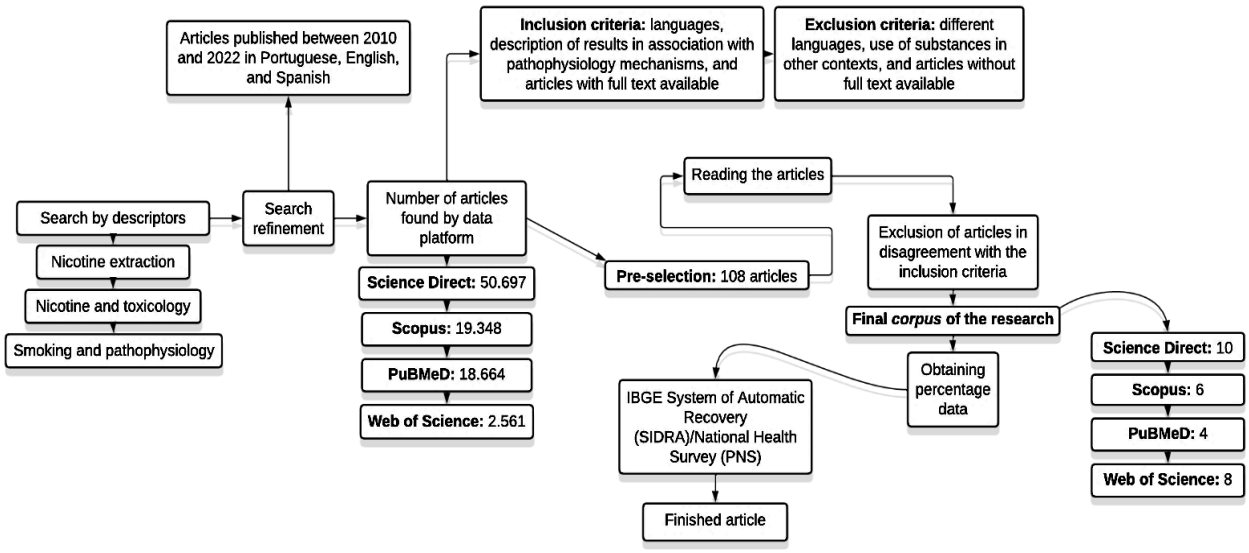

Fig. 1. Schematization of the literature review process used for the synthesis of the research.

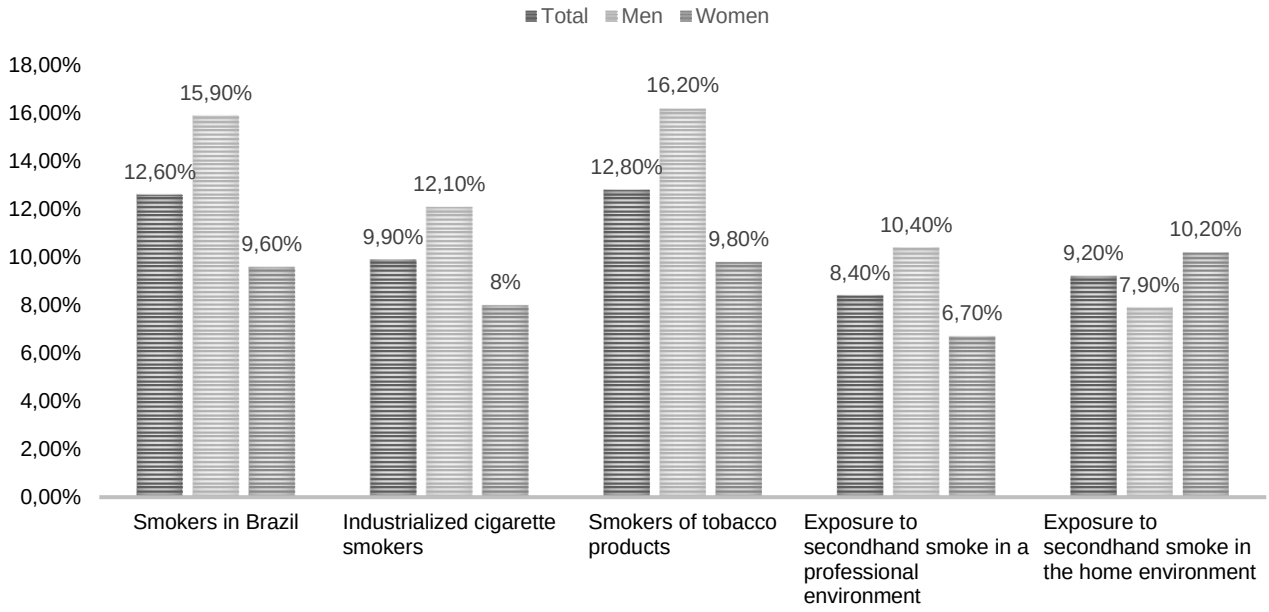

Fig. 2. Graph representative of the percentages of smoking in Brazil (2019).

3.2. Tobacco products and the production of toxic compounds

It is known that e-cigarettes, devices that work through an atomizer, rechargeable cartridge, and a battery [15], have e-liquid as a vehicle for nicotine delivery and or chemical constituents. The tobacco industry introduced the idea that such models, mainly because of their way of working, would be safer than conventional cigarettes [17]. Figure 3 shows the most commonly used forms of tobacco in the world.

A study by Tayyarah and Long [23] determined the composition of the aerosol of e-cigarettes and the smoke of conventional cigarettes to compare toxicological parameters about nicotine concentration in both forms of smoking. The average nicotine concentration per unit of e-cigarettes reached about 15.61 µg, while for conventional cigarettes 12.16 µg. Although higher concentrations of nicotine in e-cigarette units were found, for the average alkaloid content per swallow, the opposite is noted, with concentrations of 25 µg and 217 µg, respectively, representing a decrease of about 85% in the yield of nicotinic compounds in electronic devices. Thus, the trial concluded that e-cigarettes supply reduced

exposure to nicotine. However, although such research has shown and concluded that nicotinic content is exponentially lower in e-cigarettes, it is known that such devices have significant levels of volatile organic compounds (VOCs), polycyclic aromatic compounds, nanoparticles, metals, and other toxic agents [13, 24, 15, 25, 17]. For example, in the presence of metals, Pearce *et al.* [15] characterized in their study three models of e-cigarettes, with 5%, 2.4%, and 1.8% nicotine, respectively. They used inductively coupled plasma mass spectroscopy (ICP-MS) and found toxic compounds such as nickel (< 0.250 ng, 2.28 ng, and 4.97 ng), lead (> 0.050 ng, 1.06 ng and 3.28 ng), tin (< 0.100 ng, 1.06 ng, and 1.16 ng) and cadmium (< 0.050 ng for the three concentrations). It is emphasized that such concentrations refer to a total of 10 engulfed. It is noted, therefore, that in this essay, the levels of metals increased significantly while the nicotinic content was reduced, a fact that thus refutes the security propaganda portrayed by the media.

Fig. 3. Conventional, electronic and hookah cigarettes.

Besides, Goniewicz *et al.* [24] investigated the presence of carbonyl compounds, VOCs, nitrosamines, and tobacco-specific metals in 12 models of e-cigarettes. It is noteworthy that the concentrations obtained were equivalent to 150 swallows. Thus, as a result, 4 were found among the 15 carbonyls analyzed, such as formaldehyde, acetaldehyde, acrolein, and α-methylbenzaldehyde, known as toxic, irritative, and potentially carcinogenic compounds, in most samples. In addition, the great homogeneity seen between the concentrations of formaldehyde (2 to 56.1 µg), acetaldehyde (1.1 to 13.6 µg), and acrolein (0.7 to 41.9 µg) stands out. Among the 11 VOCs analyzed, 2 neurotoxic substances were detected, such as toluene (0.2 to 6.3 µg) and m,p-xylene (0.1 to 0.2 µg). *N*-nitrosonornicotine (NNN) (0.8 to 4.3 ng) and nicotine-derived nitrosamine (NNK) (1 to 28.3 ng) were the specific nitrosamines, highly carcinogenic molecules, found. Finally, for the 12 metals analyzed, cadmium (0.01 to 0.22 µg), nickel (0.11 to 0.29 µg), and lead (0.03 to 0.57 µg) were identified.

The study by Lee *et al.* [13], evaluated, through spectroscopic and chromatographic methods, whether e-cigarettes flavored tobacco and menthol affected the emission of five chemical compounds: nicotine, fractions of fine particles, carbonyls, VOCs, and trace elements. It was detected that the tobacco flavor presented an average nicotine concentration twice as high as the menthol flavor, with values of 3.13 µg/m^3 and 1.48 µg/m^3, respectively. The first flavor also presented higher concentrations of mass and many nanoparticles that easily penetrate deeply into the respiratory airways, resulting in changes in bronchial gene expression, oxidative stress, and inflammation [12]. In addition, acetaldehyde and benzene were seen at low levels; toxic trace elements such as barrio and chlorine were also found. Thus, it was concluded that the flavorings directly influence on the emission of chemical compounds in electronic devices.

El-Hage *et al.* [17] saw highly reactive molecules for the human biological system from phenolic compounds present in e-cigarette aerosols. In this study, non-substituted phenol levels and low concentrations of catechol and hydroquinone were found, substances also related to carcinogenesis and immunotoxicity, respectively. Furthermore, phenols were and are produced by reactions of addition and cyclization of smaller or intermediate molecules derived from propylene glycol, vegetable glycerin, and degradation of other products, even demonstrating that the formation of phenolic compounds in these devices is an endothermic reaction, since the intensity and duration of the swallow, factors related to the heating temperature of the e-liquid coil, were the main parameters that affected phenol emissions.

Ko and Kim [25], as well as Pearce *et al.* [15], performed the determination of the content of metals in the aerosols of e-cigarettes according to running power, also using ICP-MS. The concentrations obtained were related to 140 daily downs. In all samples analyzed aluminum (20 W = 0.007 µg and 80 W = 0.059 µg), arsenic (20 W = 0.084 µg and 80 W = 0.791 µg), cadmium (20 W = 0.097 µg and 80 W = 0.326 µg), nickel (20 W = 0.126 µg and 80 W = 0.599 µg) and lead (20 W = 0.093 µg and 80 W = 0.386 µg), metals highly toxic to human health. In addition, it is seen that at the power of 80 W, the level of metals such as lead, and nickel increased approximately 5 times. The authors concluded, therefore, that the operating power of e-cigarettes is a factor of influences the concentration of metals found in these devices.

Similarly, Neu *et al.* [14] shown, through the ICP-MS, the content of metals in a series of subsets of disposable electronic nicotine delivery systems, where 18 products of 4 different brands with 18 flavors were used. The levels found were compared with the limits of elemental impurities stipulated by the United States Pharmacopoeia (USP). As a result, they showed the presence of heavy metals such as nickel (0.52 to 11 µg g^{-1}) and lead (0.27 to 290 µg g^{-1}). It is noteworthy that the limit defined by USP for nickel is 0.5 µg g^{-1} for inhalation and 0.2 µg g^{-1} for lead, so, such metals were approximately 20 and 1.450 times, respectively, above the limits imposed by USP.

It is known that the severity of modern smoking is not necessarily in the use of nicotine, but rather in the inhalation of thousands of other toxic chemical species, ranging from metal ions to carcinogenic substances such as carbonyl compounds, volatile aromatic compounds, and tobacco-specific nitrosamines, all evidenced in the trial by Goniewicz *et al.* [24]. Together, these toxic agents alter the homeostatic process of the organism and, so, promote the development of diseases. Table 1 shows that three metals are common to the studies by Goniewicz *et al.* [24] and Ko and Kim [25]: cadmium, lead, and nickel. Such ions are also present in the trials of Pearce *et al.* [15] and Neu *et al.* [14], all related to the quantification of these compounds in e-cigarettes. Although Tayyarah and Long [23] have shown that nicotine yield is lower in e-cigarettes compared to conventional cigarettes, portraying a possible safety scenario in the use of these devices, Pearce *et al.* [15] demonstrate in their research that the levels of metals and concentration of nicotinic compounds in e-cigarettes are inversely proportional. Thus, modern smoking, supported using technological devices, is as harmful as traditional smoking to human health.

Table 1. Metallic compounds found in e-cigarettes in numerous studies.

AUTHOR	SUBSTANCES
Goniewicz et al. (2013)	Cadmium, nickel, and lead
Pearce et al. (2020)	Nickel, lead, tin, and cadmium
Neu et al. (2020)	Nickel and lead
Ko e Kim (2022)	Aluminum, arsenic, cadmium, nickel and lead

3.3. Implications of smoking on the immune system and the oncogenic process

Smoking is one of the main factors that negatively affect the immune system, leading to cellular and apoptotic changes. In these situations, the organism suffers from the imbalance of homeostasis and silent and malignant diseases begin to settle in the smoker's organism. Nicotine, although not the only one, is one of the main substances present in tobacco with immunosuppressive activity [9].

A study by Thomé *et al*. [9] investigated the association of immunological suppression of ectonucleotidase (NTPDase) and the activity of adenosine deaminase (ADA) in the lymphocytes of rats by immunosuppression with nicotine. NTPDase is an important marker of the activation of effector functions, being expressed in lymphocytes, monocytes, NK cells, dendritic cells, and T-cell subsets activated. AD, in turn, catalyzes the conversion of adenosine into inosine, being essential in differentiation and proliferation of lymphocytes. During the in vitro assay, after obtaining the whole blood and separation of lymphocytes, these leukocytes were incubated at different nicotine concentrations (5, 10, and 50 mM). In the in vivo assay, 18 animals were divided into distinct groups, one control group (using saline), one with nicotine use in a concentration of 0.25 mg/kg/day, and, finally, the last treated with nicotine 1 mg/kg/day. To ascertain cell integrity, lactate dehydrogenase (LDH) activity was used as a marker. The activity of NTPDase was determined by colorimetric assay. As a result, the activity and expression of both enzymes were altered in both trials. In the in vitro assay, with ATP as substrate, NTPDase activity was reduced by 42%,80%, and 96% at concentrations of 5, 10, and 50 mM of nicotine, respectively. In addition, inhibition was reduced when ADP was used as substrate (32%, 27%, and 49%, respectively) and ADA activity was reduced by up to 98% in the concentration of 50 mM nicotine. The in vivo assay reproduced the same results, with inhibition of both enzymes at both concentrations. It was concluded, therefore, that immunosuppression was seen due to the absence of adequate antibody responses, as well as a significant reduction in T-cell proliferation.

Although nicotine caused immunodeficiency in the trial of Thomé *et al*. [9], a survey conducted by Yu *et al*. [12] evaluated the impact of alkaloids present in e-cigarettes against the induction of DNA tape-breaking and cell death. The authors used HaCaT, UMSCC10B, and HN30 epithelial cell lines, which were exposed to e-liquids, with (12 mg mL^{-1}) and without nicotine, from 48h to 8 weeks. The results showed that the vapor of e-cigarettes without nicotine increases by up to 1.5 times the breaks of DNA tapes, while in the presence of alkaloids 2 times. Thus, it is found that DNA tape breaks are sufficiently induced even in the absence of nicotinic compounds. Furthermore, a significant increase was seen in necrotic cells and late apoptosis, where HaCaT samples exposed to nicotine-free vapor extract showed an increase of up to 68% in necrotic cells and up to 200% in secondary apoptosis. Consequently, the vapor of the e-cigarette, in the absence of nicotine, increases by up to 5 times the rate of cell death, be it by necrosis or apoptosis, and causes genotoxicity, which can lead to the development of cancer, while in the presence of nicotinic compounds, the same rate rises to 10 times. The conclusions of this study suggest that other compounds present in e-liquids, such as VOCs and carbonyl compounds, handle the observed alterations.

Although toxicological data on chemical compounds present in e-cigarettes are scarce to assess the impact of these devices on public health, Al-Saleh *et al*. [18] delimited, for the first time, DNA damage, chromosome breakage, and cell viability in human and animal cells through the comet, micronucleus, and triptan blue assay, respectively. An amount of 68 e-liquid refills of 33 different brands with nicotine content between 1 and 8 mg were used. The chemical profile of e-liquids was analyzed by GC-MS, while cytotoxicity and genotoxicity were investigated by exposure of TK6 human lymphoblast strains and Chinese hamster ovary (CHO) cells. Most e-liquids were shown to induce DNA damage, chromosomal breakage, and cytotoxic effects. The authors pointed out that the DL-menthol flavor, even at low levels, is associated with reduced cell viability and increased genotoxicity, however, concluded that this data should be adequately investigated because the flavoring is commonly used in electronic devices.

Figure 4 illustrates one of the immunosuppression mechanisms caused by nicotine and the general principle of cytotoxicity and genotoxicity associated with this alkaloid.

Cytotoxicity associated with genotoxicity can lead to the development of neoplasms, including malignancies. It is known that conventional cigarettes are one of the main nicotine delivery products responsible for such changes, however, Yu *et al*. [12] and Al-Saleh *et al*. [18] confirmed that e-cigarettes are also capable of damaging cells and their genetic material.

Jacob *et al*. [20] compared, through a randomized crossover study, the toxic substances in the smoke of hookah and traditional cigarettes aiming to verify the carcinogenic potential of these products. For this, 13 volunteers, for 4 days, were separated into 2 groups. One of the groups took part daily in 3 hookah sessions, while individuals in the second group smoked 11 cigarettes per day. Biomarkers of exposure to various toxic substances were measured, such as nicotine, carbon monoxide (CO), NNAL (metabolite of NNK), polycyclic aromatic hydrocarbons (PAHs) and metabolites of mercapturic acid of various VOCs. Plasma nicotine concentrations were lower during hookah use as well as NNAL levels, however, expired CO in hookah was 86 ppm, and in industrialized cigarettes 5.2 ppm. PAHs metabolites found for hookah were 1-hydroxypyrene, while for cigarettes, 2-naphthol and 1,2,3-hydroxyfluorenes. Furthermore, hydroxyphenantrene excretion was similar for both groups. For VOCs metabolites, the use of hookah generated greater excretion of phenylmercapturic acid, a metabolite of benzene; metabolites of ethylene oxide, acrylonitrile, acrolein, propylene oxide, and 1,3-butadiene were higher in cigarette smoke. In this study, it was noted that pyrene and phenanthrene levels in hookah were high, with values of 81 pmoL/24h and 296 pmoL/24h, respectively.

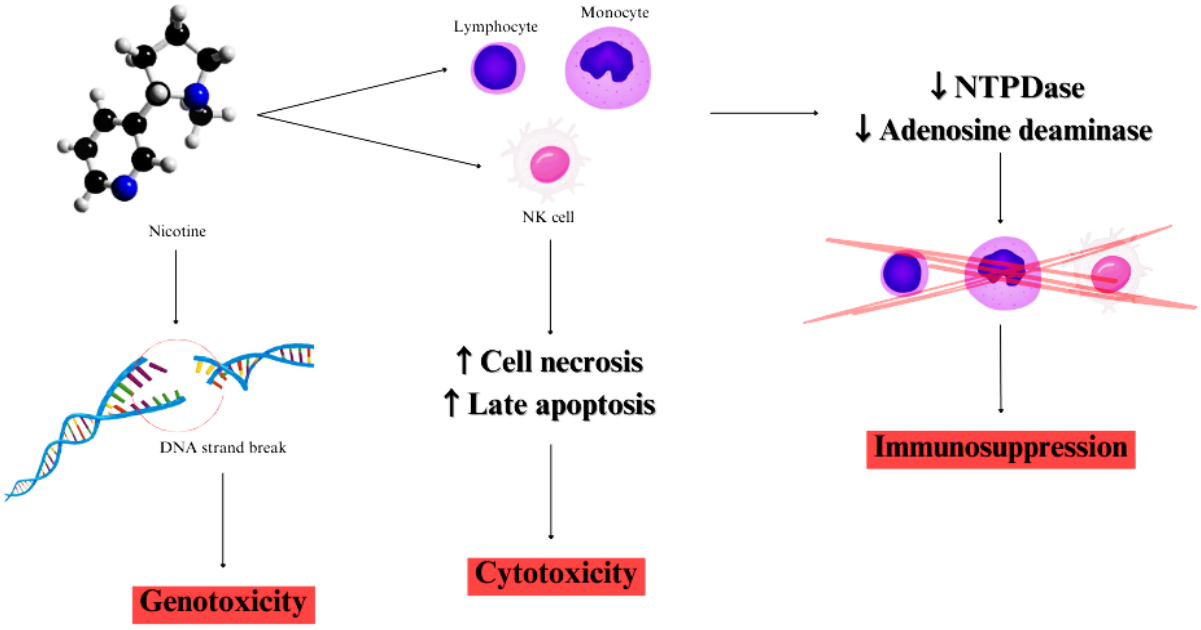

Fig. 4. Nicotine-related mechanisms of genotoxicity, cytotoxicity and immunosuppression.

A survey by Luo *et al.* [26] used deuterated phenanthrene to trace the critical profile of PAHs metabolic pathways in 170 smokers and 57 non-smokers. The results found whether the effect of smoking is on metabolic activation or phenanthrene bioinactivation. The smokers had a higher proportion of activation products, such as [D10]1,2-PheD, and a lower proportion of bioinactivation products, such as [D9]OHPhe. Thus, it was concluded that smoking causes an increase in the metabolic activation of phenanthrene, mainly by the bioactivation of CYP1A1, an enzyme activated up to 100 times by smoking. It is note point that phenanthrene has structural and metabolic pathway similarities with benzo[α]pyrene (BaP), a highly oncogenic compound, as shown in Figure 5. Thus, the final product of phenanthrene activation is strongly correlated with BaP-tetraol, thus associating with lung cancer.

In addition to lung cancer, one of the best-known complications of smoking, renal and pancreatic cancer also stand out as grave consequences resulting from chronic smoking. Renal cell carcinoma (RCC) is one of the most prevalent histopathological forms of kidney cancer, a malignant neoplasm that increases the chances of developing in smoking men by 50% and 20% in female smokers, mainly because nicotine has renal excretion [27].

To find the carcinogenic potential of chronic nicotine exposure, Chang and Singh [27] treated human renal proximal epithelial cells (HK-2) with 1 and 10 μM of tobacco alkaloid. The authors mainly evaluated cell growth, morphological changes, and molecular transformations using the MTT test (3-(4,5-dimethyliazole-2il)-2,5-di-phenyl tetrazolium bromide), western blot test, flow cytometry immunophenotyping and quantitative reactions of reverse transcriptase-PCR (qRT-PCR). As a result, the cells exposed to 1 μM of nicotine increased by 61.2% and those treated with 10 μM increased by 11.9%. Also, among the main morphological alterations, we observed the activation of the AKT and p38 pathway, as well as a significant increase, in a dependent dose, of the biomarker of DNA damage, that is, an early biomarker in oncogenic transformation (pH2AX), indicating that, in transformed cells, there is an accumulation of DNA breaks.

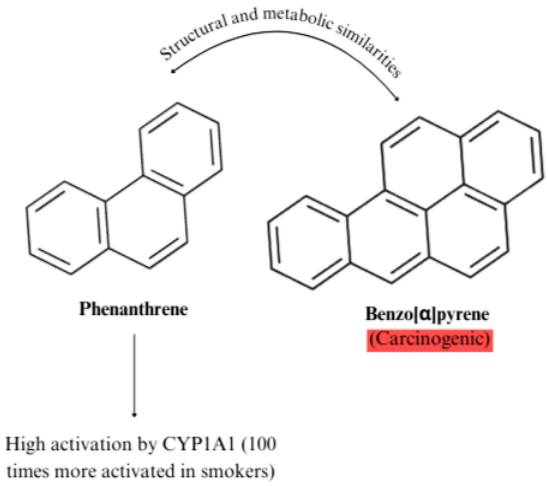

Fig. 5. Phenanthrene and benzo[α]pyrene.

Therefore, as a conclusion, they highlighted that, biologically, low concentrations are chronically more potent in inducing transformation into HK-2 cells. Nevertheless, nicotine is still capable of causing hypermethylation of tumor suppressor genes in pancreatic cell lines, as showed by Jin, Hao, and Fan [28].

Thus, it is worth mentioning that such a change in methylation patterns leads to the inactivation of these genes – a phenomenon seen in pancreatic cancer, a fatal neoplasm, mainly due to its lack of response to routine chemotherapy. The researchers also noted increased proliferation and inhibition of apoptosis in these cells, changes commonly saw at the beginning of carcinogenesis.

3.4. The respiratory system as a target of smoke exposure

One of the main pathogens that affect the lung of smokers is Chronic Obstructive Pulmonary Disease (COPD), with consequent narrowing of the airways, reduction of the surface area for gas exchange, and, therefore, loss of elastance and pulmonary compliance. Given this, Zeng *et al.* [7] investigated

the interaction and regulation mechanism of USP7, a deubiquitinant enzyme, and p300 using conventional cigarette smoke extract in endothelial progenitor cells of patients, and models of mice, with COPD. The authors saw interactions between USP7 and p300, with consequent p300 instability. They also found that in the cell culture medium, the p53 level increased in a dose-dependent manner, showing that tobacco smoke extract can regulate when linked to USP7-p300, the concentration of this protein at the post-transcriptional level. In mice, there was positive regulation of the p21 protein through the USP7-p300-p53 route. They concluded, therefore, at the end of the study, that tobacco smoke extract can generate abnormal p300 accumulation in endothelial progenitor cells, positively regulating USP7 expression. As soon as the interaction between both proteins occurs, there is, then, excessive activation of the p53-p21 signaling pathway, resulting in the cell cycle stopping by inhibiting the activity of cyclin-dependent kinases (CDKs). This may be one of the causation mechanisms of COPD in smokers [7].

It is emphasized that COPD does not develop only in traditional smokers, in the studies of Rammah *et al.* [19] changes in the profile of type II alveolar cells, such as A549, and in endothelial cell lines during their exposure to hookah smoke were seen. It was found that, in addition to the smoke of the device behaving like a weak mutagen, it inhibited cell proliferation, through a stop in the cell cycle, at a dose of 4 mg mL^{-1} and caused cytotoxicity at the dose of 8 mg mL^{-1}. Just like Zeng *et al.* [7], Rammah *et al.* [19] showed an accumulation of p53 and p21. Once such changes occurred, there was, therefore, induction of cellular senescence. Senescent cells, therefore, increased the production of matrix metalloproteinases (MMPs), enzymes that stimulate tissue destruction, and toll-like receptor 4 (TLR-4), which plays a role in pulmonary integrity. Among the MMPs, MMP-2 and MMP-9, the main elastolytic MMP, were the most found. The authors concluded that hookah is a risk factor for the development and progression of COPD, because patients with such pathogenesis present alterations in cell growth, with abnormal repair mechanisms given cell cycle stop and, increased levels of MMP-2, MMP-9, and TLR-4, that is, the same modifications induced by hookah smoke.

A study by Ginzkey *et al.* [10] proved, through the comet assay in the alkaline version, that nicotine causes, through nACh receptors, genetic damage in human pulmonary epithelium cells (BEAS-2B). In addition, direct losses such as induction of base modification, cross-links, and single and double tape breaks were detected. The research showed that, in the presence of mecamylamine, a non-competitive antagonist of nAChRs, there was a significant reduction in genetic damage, that is, tobacco alkaloid needs their receptors to trigger genotoxic effects on lung tissue. Furthermore, they seen that such effects are mainly associated with oxidative stress because when *N*-acetylcysteine, an antioxidant, was added to the medium, genotoxicity was prevented.

Previously Pearce *et al.* [15] concluded that the power of the e-cigarette directly affects the concentration of metals in these devices. Similarly, Otręba *et al.* [4] perceived that the cytotoxicity of e-cigarette aerosols is interconnected with high voltage and battery, and with the addition of flavor and/or nicotine to e-liquids. In any case, it is seen, with the aid of the colorimetric assay WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium)-1,3-benzene disulfonate), in these parameters, a significant reduction in the viability of alveolar lung cells type II (Figure 6).

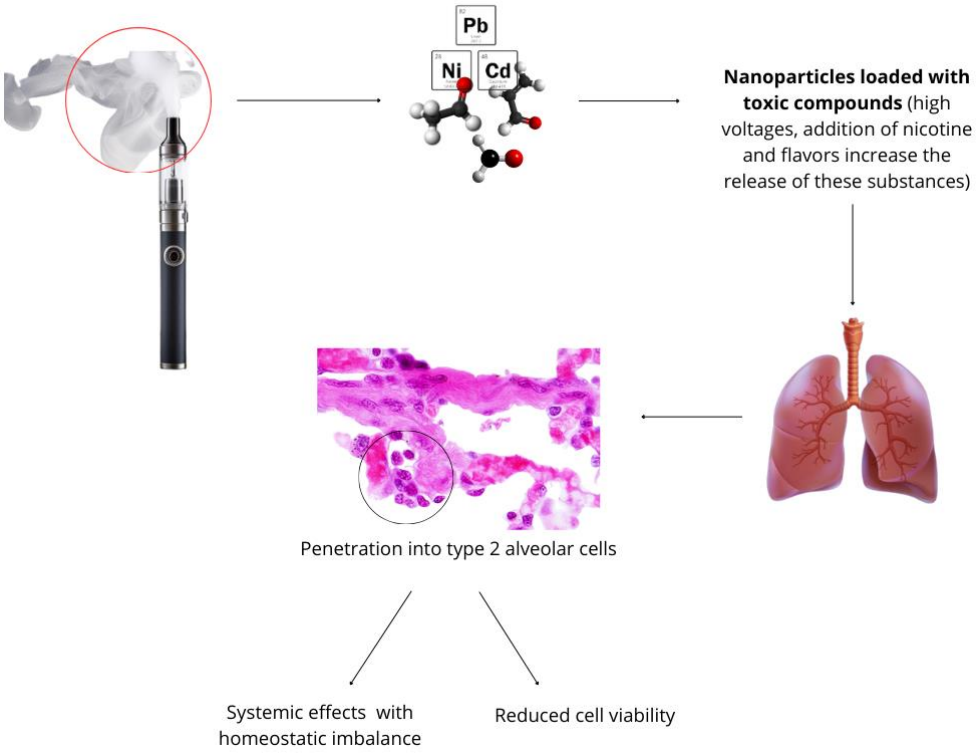

Fig. 6. Nanotoxicity of e-cigarettes.

To verify the impact of nicotine on infection by SARS-CoV-2, Maggi *et al.* [8] exposed A549 cells to the alkaloid and later analyzed, through RNA extraction and qRT-PCR analysis, possible changes in the expression of ECA-2, a known receptor for coronavirus. The cell lines were treated with 0.1 μM of nicotine for 24h and were then submitted to simple-tape

DNA extraction by the TRIzol reagent, with consequent analysis of reverse transcriptase aiming to observe mRNA particles related to ECA-2. A sharp increase in enzymatic expression was detected, with a peak after 24h and a decline from 48h. Consequently, there was an induction in the replication of SARS-CoV-2. It is noteworthy that the virus spike protein interacts with ECA-2 aiming at the entry of the infectious agent into the pneumocytes. Thus, the authors concluded that acute smoking may be a risk factor for severe conditions of Covid-19.

3.5 Compromising the cardiovascular system in smokers

Smoking is one of the determining factors of cardiovascular system involvement, making smokers more likely to suffer serious incidents, such as acute myocardial infarction (AMI) and/or ischemic stroke (CIA). One of the primary alterations seen in individuals who have a damaged cardiovascular system is the presence of atherogenic plaques in arteries and blood vessels of considerable caliber. Atherosclerosis results mainly from endothelial dysfunction – a process generated by nitric oxide (NO) deficiency due to endothelial NO (eNOS), tetrahydrobiopterin (BH4), and GTP cyclohydrolase 1 (GTPCH1) [6].

Thus, to study the association of nicotine with endothelial dysfunction, Li *et al.* [6] investigated whether GTPCH1 behaved as the target of HuR, a protein that regulates the stability of mRNA, as a plasminogen 2 activator inhibitor and endothelial growth factor and whether the negative regulation of GTPCH1 in endothelial cells could mediate nicotine-induced endothelial dysfunction. They saw that the level of GTPCH1 was reduced by the nicotinic compound because of the suppression of HuR translocation. Consequently, as expected, BH4 and NO levels also decreased. It should be noted that, when BH4 is low, eNOS begins to add molecular oxygen to the reduced dinucleotide of nicotinamide-adenine instead of L-arginine, which leads to the production of ROS – a phenomenon seen in vitro by the authors. Thus, in addition to endothelial dysfunction caused by the low bioavailability of NO, nicotine can generate lipid peroxidation due to the performance of ROS. These same results were seen in a prospective cohort study based on the Gutenberg Health Study, which included the analysis of 15.010 participants between 2007 and 2012 to investigate the association between smoking and endothelial dysfunction. Exposure to smoking was associated with a worsening of endothelial dysfunction and cessation of an improvement in arteriolar endothelial function. It is emphasized that endothelial dysfunction precedes the development of thrombotic and atherosclerotic processes due to changes in physiological vasodilation that, so, culminate in increased systemic blood pressure, a metabolic disorder that directly affects the cardiovascular system [1].

Another event that precedes a critical condition related to cardiovascular diseases is pathological platelet aggregation, which may generate, for example, Deep Vein Thrombosis (DVT). In this way, Nocella *et al.* [5] conducted a single-blind cross-sectional study between September 2014 and March 2015, in 40 healthy individuals (20 smokers and 20 non-smokers), to ascertain the degree of platelet aggregation in smoking conventional cigarettes and e-cigarettes. In the first phase, all participants smoked an industrialized cigarette with an average nicotine content equivalent to 0.6 mg and after one week, in the second phase, there was vaporization of an e-cigarette flavored tobacco. It is noteworthy that for e-cigarettes, individuals vaporized only 9 puffs to equal 0.6 mg of alkaloid. For each phase of the study, blood samples were collected before and 5 minutes after smoking, aiming to measure platelet activation biomarkers. As a result, the authors concluded that the use of both products generated an acute increase in platelet activation, where soluble forms of CD40L and P-selectin increased significantly in smokers and nonsmokers. In non-smokers, conventional cigarettes generated an increase of about 32% in platelet aggregation, while in smokers, activation was less expressive, with an increase of 16%. Finally, during e-cigarette smoking, platelet aggregation in nonsmokers increased by 11%, while in the other group, only 9%. These results should be thoroughly analyzed in further studies. In any case, the authors concluded that nonsmokers who intend to use e-cigarettes should be discouraged, mainly for recreational purposes, because, as evidenced, smoking can generate and/or aggravate disorders to physiological platelet function, increasing the risk of developing DVT.

Ren *et al.* [16] also showed the harms of e-cigarettes on blood metabolites and damage to cardiac tissue. Mice were exposed to e-cigarette smoke (3% nicotine) for up to 8 hours and, after two weeks, had cardiac tissue and blood removed for histopathological and biochemical analysis, respectively. Regarding cardiac tissue, the 8-hour group had interstitial edema, with an apoptosis rate 10 times higher. Evidence of oxidative stress was also detected. In the blood, an increase in total cholesterol and triglycerides was seen, according to the prolonged exposure time. Such information shows that the smoke of e-cigarettes can lead to the development of hypercholesterolemia, hypertriglyceridemia, or, even, mixed hyperlipidemia, as well as affecting the homeostasis of the cardiovascular system through damage to cardiovascular function.

3.6 Passive smoke as a risk factor for attic homeostimbalance

Passive exposure to cigarette components can occur through ingestion, inhalation, or even skin, generating damage like active smoking in the body of the exposed individual. Secondhand smoke handles accounting for 10% of cancer-related deaths. This is due to PAHs and nitrosamines, highly carcinogenic substances that are present in cigarettes, and due to their presence in smoke reaching people passively [2].

Nicotine that is retained on surfaces can react with atmospheric ozone and nitrous acid, generating the formation of 1-(*N*-methyl-N-nitrosamine)-1-(3-pyriride)-4-butanal (NNA), NNK, NNN, formaldehyde, n-methyl formamide, cotinine, and other compounds – in this way, it has to be that, the older it is smoke, the greater the toxicity of chemical agents [3]. It is noteworthy that nicotine is extremely difficult to remove from surfaces and should therefore be removed with the aid of acidic substances [29], which makes the scenario even more troubling.

A study by Schick *et al.* [3] aimed to develop a system to age cigarette smoke, with ozone injections aimed at replacing the concentrations of this gas present in atmospheric air, aiming to reproduce the chemical changes that occur with nicotinic compounds. The concentrations of PAHs, nicotine, cotinine, and nitrosamines were determined before and after the aging process generated using a 6 m^3 stainless steel aging chamber, which contained three vertically staggered baffles and two internal ventilators. Cotinine, a stable nicotine oxidation product, was the only substance that did not have its concentrations reduced as tobacco smoke aged. NNK was 10 times more present than nicotine in the analyzed samples,

a result suggestive that tobacco alkaloid reacts with atmospheric ozone because the reaction with oxygen is inhibited in acidified solutions, so only the free base is susceptible to oxidation to form the carcinogenic compound. The authors concluded that 70% of nicotine and 80% of tobacco-specific nitrosamines are deposited on surfaces and are not removed under normal ventilation conditions.

A study repeatedly exposed cotton and polyester fabrics to cigarette smoke to ascertain the degree of impact of active smoking indoors. After exposure, both tissues were folded and stored in polyethylene bags at room temperature in the dark. The analyses were performed using liquid-mass tandem chromatography (LC-MS/MS). As a result, it was found that passive smoke chemicals remained for more than 12 months after the last exposure. Nicotine, like nitrosamines, was quickly extracted from cotton tissue in an aqueous medium of composition like saliva and sweat – this shows that, for example, if a child bites a cloth that was previously exposed to cigarette smoke, it will suffer, consequently, significant exposure to chemicals. Furthermore, regarding the differences in the concentrations of components of cigarette smoke, it was observed that cotton tissue, after 5 months, underwent a multiplication of NNK levels (169.5 ng g^{-1} – 218.8 ng g^{-1}), an increase that was also detected for NNN (37.10 ng g^{-1} – 45.84 ng g^{-1}), nicotelin (105.8 ng g^{-1} – 113.22 ng g^{-1}) and nicotine (105.8 µg g^{-1}) and nicotine (105.8 µg g^{-1}) and nicotine (105.8 µg g^{-1} – 112.92 µg g^{-1}). In polyester tissue, concentrations were significantly lower after 7 months, with about 0.557 µg g^{-1} in the first month and 1.689 µg g^{-1} in the seventh month for nicotine, 36.35 and 59.61 ng g^{-1} for nicotelin and 3.2 ng g^{-1} for NNK, which had significant concentration only after the seventh month. Such differences can be explained by surface chemistry, where cotton, unlike polyester, has three free hydroxyl groups and glucose monomers, which can form hydrogen bonds with the cigarette alkaloid. In any case, it was concluded that the tissues of the internal environment function as reservoirs of chemicals of third-hand smoke [2].

After 2 years, Bahl *et al.* [30] evaluated by cytotoxicity and genotoxicity assays whether passive smoke can affect the health of individuals exposed to third-hand smoke. For this, a scenario had been simulated in which 20 cigarettes were smoked daily, so that a cigarette was smoked for 1 minute, every 2 hours, for 8 hours, for 30 days. The evaluation of cellular toxicity, measured by the conversion of a tetrazolium salt into a colored product through the enzymatic activity of metabolically active compounds (MTT assay), indicated higher cytotoxicity in cells treated with smoke extracts associated with saline, a factor that indicates that the presence of serum proteins, such as albumin, found in saliva, aid in the extraction of cytotoxic compounds present in extract cigarette smoke. The authors also detected inhibition of cell proliferation and genotoxicity, as certain by single-cell gel electrophoresis. It is noteworthy that DNA damage can be explained by the presence of NNK, NNN, and NNA, substances that were found in cellular tissues.

E-cigarettes are devices that supply damage the body of passive smokers. A survey by Goniewicz and Lee [29] evaluated nicotine deposition on surfaces (windows, walls, flooring, and metal and wood surfaces) as a third-hand exposure marker to e-cigarettes. For this, e-cigarettes refilled with e-liquid orange, mandarin, gum, and tobacco were used, with nicotine concentration at 24 mg mL^{-1}, 32 mg mL^{-1}, and 20 – 24 mg mL-1, respectively. The environmental samples were analyzed by gas chromatography with a nitrogen-phosphorus detector. As a result, three of the four experiments detected an increase for nicotine on all surfaces, focusing on glass and floor windows, with an increase in the alkaloid concentration of approximately 50 µg/m^2 and 200 µg/m^2, respectively.

Aiming to highlight the passive risk generated by the chemical emissions of e-cigarettes, Offermann [11] performed the exposure of a non-smoker for 8 hours to 125 swallows of e-cigarettes. Direct smoking analyses identified that 4 of the 9 products analyzed exceeded the NSRL (No Significant Risk Levels) risk quotient imposed by the California Office of Environmental Health Hazard Assessment, whose value was set at 1.0 for cancer-related health events such as lead metals (1.33) and cadmium (5.13), and other toxic substances such as formaldehyde (1.64) and NNK (2.36). Furthermore, 2 chemicals exceeded the CREL (Chronic Reference Exposure Levels) risk quotient of 1.0 for non-cancer health effects, such as nicotine (222) and propylene glycol (967). As a comparative parameter, they saw that indirect smoking also had a high CREL risk quotient for nicotine (5.4) and propylene glycol (23). In this sense, it is seen that, although secondhand smoke from e-cigarettes does not have such an abrupt impact on the health of the exposed individual when compared to active smoking, it demonstrates considerable toxicity to the human biological system.

Thus, as a conclusion of both studies, it is found that e-cigarettes, as well as conventional cigarettes, generate the surface deposit of nicotine and are also responsible for the consequences of secondhand smoke.

4. Conclusions

Although conventional cigarettes manage greater harm to human health, e-cigarettes also present toxicity. Vigorous regularizations should be put on the agenda, not only by the presence of carcinogenic compounds in the smoke of its aerosol but also by the frequent detection of considerable levels of heavy metals in its e-liquids.

Furthermore, the idea that associates hookah with a recreational process should be eliminated, because this device, in addition to harming the respiratory system and restricting airflow, generates higher concentrations of monoxide carbon than the conventional cigarette itself and behaves as a factor for mutagenicity. Nevertheless, the new findings about the impact of third-hand smoke, and smoking, as a public health problem, deserve more attention, especially on the part of tobacco users. Prudence should be redoubled in home environments and other closed places, since nicotinic compounds are hardly eliminated under normal conditions, thus affecting non-users.

Finally, it is concluded that none of the forms of smoking has adequate safety, not seeing them interchangeable with each other, either in the search for the least toxic device or even aiming at cessation pseudo therapies.

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