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REVIEW ARTICLE

The safety of using oral nicotine pouches – consideration of their effects on general health, oral mucosa and periodontal diseases, comparing them to snus and other nicotine-containing products

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Abstract

Introduction and Objective. Oral nicotine pouches (ONPs) are tobacco-free and non-combustible products that are pouch-shaped and fit between the alveolar process and the upper lip. Through their use, nicotine is absorbed into the body through the oral mucosa. Their harmfulness is significantly less than that of traditional cigarettes because they do not require combustion.

Review Methods. A review was carried out of the literature in English from 2010–2023 using the PubMed database.

Brief description of the state of knowledge. Research indicates ONPs have lower cytotoxicity compared to snus and cigarettes, with some studies suggesting reduced harm-related biomarkers. However, they still pose health risks. The unregulated status and accessibility of nicotine pouches, especially to youth, underscore the need for awareness of their potential dangers. It is important to emphasize their potential negative effects on the oral mucosa and periodontium, as well as on the body, due to their content of nicotine and other potentially dangerous substances. ONPs are suspected of contributing to mucosal lesions, gingival recession, alveolar bone loss, and increased mediators of periodontitis like MMP-1, MMP-3, and IL-1. Application of ONP to human gingival epithelial cells (HGEPp) resulted in elevated levels of lactate dehydrogenase (LDH), ROS, and inflammatory cytokines (TNF-α, IL-6, and IL-8). ONPs contain low levels of tobacco-derived carcinogens, and may include substances classified by the IARC as potentially carcinogenic.

Summary. Due to the short time the product has been on the market and narrow period of widespread use, the effects of ONP on human health cannot be predicted with certainty. It is very important to conduct further research in several areas regarding the health effects of ONP and potential consequences that may occur in the future.

Key words

addiction, inflammation, risk, bacteria, teeth, toxicity, periodontium, snus, oral nicotine pouches, nicotine pouches and mucosa

INTRODUCTION

Public awareness of the harmful effects of cigarettes on health has increased significantly over the past few decades. However, this has been accompanied by a search for alternative nicotine delivery methods to reduce the risk of developing smoke-related diseases. For this reason, tobacco-free and non-combustible products like e-cigarettes, tobacco heating products (THPs), snus, and oral nicotine pouches (ONPs) have become increasingly popular on the market. It should be noted that they are particularly popular among young people [1]. These methods of nicotine intake are being considered to help reduce the number of adults who smoke and encourage them to quit [2]. In particular, their successful marketing campaigns promote these products as having minimal harm and being a lower-risk alternative; however, a number of studies have highlighted the harm they may cause. Therefore, it is important to monitor new nicotine-containing products carefully in the light of the limited evidence of their safety.

Oral nicotine pouches should be distinguished from Swedish snus, even though they are sometimes confused due to their similar shape and method of consumption. Both products are pouch-shaped and fit between the alveolar process and the upper lip. Rather than the smoke from cigarettes, warming tobacco heaters, or E-cigarettes, nicotine is absorbed into the body through the oral mucosa. Research findings [3] suggest that the substance is absorbed into the blood through the oral mucosa, rather than absorption through the gastrointestinal tract. This method is beneficial for its ease, delightful flavour, and subtlety. The key distinction is in what is inside the pouches. Snus is a moist or semi-soft oral product of ground tobacco plant, either loose or in a pouch, ONP is light in colour, not brown. Many pouches are typically offered in small plastic containers [4, 5].

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Figure 1. Typical nicotine pouch

Figure 2. Product in container.

Figure 3. Method of consumption

Emergent non-tobacco oral nicotine products are largely composed of water and microcrystalline cellulose, which combined comprise approximately 80–90% of the ONP. The outer pouch material is composed of microcrystalline cellulose fibres that have been chemically, thermally, or solvent-bonded together. This matrix also contains sodium chloride, water, humidifying agents, a pH buffer – sodium carbonate, a filling agent, taste enhancers and flavourings, sweeteners, and pharmaceutical grade nicotine salt, all of which are food-grade standard components. They are commercially available for less than ten years in a variety of nicotine flavours (e.g., peppermint, spearmint, liquorice, citrus, berry) and strengths [4, 5]. These items are made to be placed between the upper lip and gum, providing a steady release of nicotine for 30 – 60 minutes with each application. The lack of tobacco is likely to ensure ONP has a lower risk profile than snus [6].

Determining whether to feel excited or sceptical about the rising popularity of smokeless nicotine products is challenging. Arguments exist for and against the trend of using ONP. A major advantage is their significantly lower harm compared to traditional cigarettes due to the absence of tobacco combustion. Epidemiological evidence already suggests a lower incidence of cancer compared to smoking. Snus use is believed to entail only a 1% risk compared to smoking traditional cigarettes [6]. Additionally, the rate of tobacco usage in Sweden is similar to that of other European countries, but the mortality rate related to smoking is significantly lower than in Europe. Snus use is popular in Sweden and has contributed to the lowest smoking prevalence rates in Europe [4]. As of 2017, Sweden had a daily cigarette smoking prevalence of only about 5%, which is significantly lower than the average of 25% in Europe. On the other hand, 20% of Swedes reported using snus every day. This phenomenon, usually called the 'Swedish experience', is a significant aspect in the discussion of public health concerns [5, 7, 8].

For these reasons, nicotine pouches can be considered a reasonable alternative to smoking for individuals who are not ready to completely overcome their nicotine addiction, as it shares a similar nature of usage with other non-combustible nicotine products. They could be an initial step towards change, potentially leading to medically recommended nicotine replacement therapy (NRT), such as Nicorette® lozenge and Nicotinell® gum [9]. It should be noted that ONPs are not currently classified as therapeutic treatments.

A screening was conducted to assess 43 harmful and potentially harmful substances in two variations of the nicotine pouch product ZYN. The results showed that only a few of these substances were detectable and that their levels were consistently low. These results closely align with the outcomes obtained from evaluating NRT products using the same method. It is noteworthy that neither nitrosamines nor polycyclic aromatic hydrocarbons were identified in either the ZYN or NRT products. This investigation suggests that there is a similarity in the potential harm between NRT and ONPs. However, it is important to note that the study was funded by a tobacco company [9]. An analogous study investigated 26 different chemicals found in various nicotine delivery systems, including a Lyft Nicotine Pouch [5].

Debates persist on ONPs as a safer substitute for snus, with some studies showing lower cytotoxicity. They may present lower health risks than snus when smokers exclusively switch due to their lack of tobacco, fewer harmful substances, and reduced toxicants and daily intake. According to research conducted by Azzopardi et al., estimates of exposure and toxicant composition suggest that nicotine pouches may have a position between Swedish snus and NRTs [5].

Data shows that levels of exposure biomarkers, except for total nicotine equivalents, significantly decrease in nicotine pouch users compared to smokers. Moreover, the

majority of biomarkers associated with potential harm indicate statistically significant improvements or are on par with individuals who have never smoked or are former smokers [10]. In contrast, as mentioned in previous studies, the cytotoxic impact of ONPs may be similar to or different from snus, and it could be even larger. This depends on the specific flavours present in both ONPs and snus products.

Regarding the disadvantages and risks of widespread nicotine pouch use, it is crucial to highlight their unregulated legality and easy accessibility to young individuals. According to studies, the most prevalent among adolescents is the use of e-cigarettes, while the consumption of nicotine pouches is less frequent. However, due to marketing and appealing flavours, the use of nicotine pouches is on the rise [1]. Nearly half of young tobacco users reported using multiple tobacco products, and the majority of them expressed a preference for flavoured options [11]. ONPs are attractive to consumers because of their simplicity of use, unobtrusiveness, absence of smoke odour and availability in a range of flavours. The subtle nature of pouches make them appealing especially to teenagers because they can use this form of nicotine without being detected by parents or caregivers. Nicotine pouches can quickly lead to addiction among individuals who may not have otherwise started smoking. This is due to the wide range of flavours and marketing approaches that specifically cater to the younger demographic. Nicotine exposure during adolescence can have harmful effects on brain development, leading to an increased risk of nicotine addiction and causing difficulties with attention, memory, and learning [1].

Similarly, among adults this form facilitates regular use without leaving workplaces, indoor areas, or public spaces. People often use combustible products like e-cigarettes, tobacco heaters, and conventional cigarettes alongside nicotine pouches. With respect to flavoured tobacco, it was strongly observed that the use of these products was correlated with a significantly higher likelihood of consuming two different types and multiple varieties of tobacco products, in comparison to those who only partook in a single kind, even after taking into account various influencing factors [11]. Both of these factors may contribute to increased nicotine intake frequency and higher daily consumption levels. Depending on a person's motivation, nicotine replacement therapies (NRTs) can help individuals quit smoking, but they can also maintain the addiction in a less harmful manner [12].

Well-known manufacturers of pouches include Zyn (Swedish Match), On! (Altria), and Velo (R. J. Reynolds). Their products are widely available in the mass market, although not in every country [13]. Since 2016, ONPs have been available for purchase, starting with the US and followed by Europe in 2018 [14, 15].

The Tobacco Products Directive 2014/40/EU (TPD) does not currently apply to tobacco-free nicotine pouches. Snus and other oral tobacco products are no longer allowed to be sold in the EU, with the exception of Sweden. However, comparable products that do not include tobacco but do contain nicotine – including those with extremely high doses – are exempt from this restriction. Examples of these products are nicotine pouches, which are primarily marketed by big tobacco corporations. In response to its recent surge in popularity, member nations have adopted wildly divergent regulatory stances, ranging from outright prohibition to unrestricted sale [16]. At the moment, pouches are not widely accessible in all European countries, but this is constantly evolving. In Poland, they are exempt from excise tax, labelled as a foodstuff (most EU member states regulate nicotine sachets as a consumer product), and can be found in various outlets such as online stores, chain stores, petrol stations, and tobacconists. Besides Poland, they can be obtained in other EU countries, such as Sweden, Denmark, Italy, Latvia, Estonia, and outside of the EU, in the UK and US. Some of the countries, such as Sweden, Denmark, the Czech Republic and Slovenia, have introduced special regulations for nicotine pouches. The EU does not yet have standardized legislation for this category of products [17].

Nicotine pouches, an unregulated emerging product, raise concerns about health risks and addiction due to their easy accessibility without oversight. Lack of regulatory warnings allows unrestricted purchase and consumption, with no specified maximum nicotine content or dosage warnings. Debates in Poland center on implementing health warnings, consumer information, age restrictions, and possible taxation under nicotine-containing, tobacco-free product categories.

OBJECTIVE

The aim of the review was to offer a summary of the features of a novel nicotine product, evaluate the potential negative effects on the oral mucosa and periodontium, and compare them to snus and other nicotine-containing items, as well as showcase the existing understanding of the effects of nicotine pouches on health and emphasize the importance of monitoring. Additionally, the review attempts to pinpoint possible regions of enquiry that necessitate additional examination.

REVIEW METHODS

A review was carried out of the literature in English from 2010 – 2023 using the PubMed database. The database was searched using the key words: oral nicotine pouches/ nicotine pouches and mucosa/snus/toxicity/periodontium / inflammation/addiction/risk/teeth/bacteria.

RESULTS

While the adverse effects of smoking are extensively documented, it is crucial to acknowledge that the majority of smoking-related diseases do not result directly from nicotine. Instead, the harm is primarily attributed to toxic compounds present in inhaled tobacco smoke. Data reveals that using ONPs leads to statistically significant declines in biomarkers linked to carcinogens, respiratory toxicants, cardiovascular toxicants, reproductive or developmental toxicants, and urine mutagenicity, as opposed to smoking [18]. Adult smokers may view nicotine pouches as a viable alternative for achieving the desired nicotine intake. Taking into account the pharmacokinetic characteristics and research parameters, it is unlikely that these medications will be abused and the risk of addiction is low [3]. It has been found that nicotine pouches take a longer time (60–65 minutes) to reach the peak concentration of nicotine in the bloodstream, compared to cigarettes, which only take seven minutes. In terms of personal experiences, the ratings for enjoyment and the likelihood of

using the product again were higher for cigarettes compared to all other nicotine products. The ONP with the lowest nicotine content received the lowest ratings [19].

Nicotine, albeit relatively secure at the concentrations detected in tobacco [5], is not completely innocuous to human tissues. Evidence suggests that using smokeless tobacco products could cause changes to the mucous membranes, evidenced by small, white spots appearing above the teeth where snus or nicotine pouches are placed [20]. Therefore, it is imperative to comprehend the effects of oral nicotine pouches products on oral mucosa, given their nicotine content and the presence of other chemical substances. Equally with chewing tobacco, smokeless tobacco, and snus, users of ONPs position the pouches between their lip and gum. According to a study, approximately 20% of young snus users experienced gum recession, caused mainly by their addiction. The quantity of snus consumed and the duration of snus application were linked to more severe lesions and gingiva retraction, respectively [21].

Local gingival recession is the most common type of irreversible oral mucosal injuries caused by snus. They usually appear at the location where a snus is applied [22]. Placing the pouches in close proximity to the gingival tissue may potentially result in irritation and mechanical trauma [23, 24]. Leukoedema and hyperkeratotic lesions of the oral mucosa are examples of non-cancerous oral lesions caused by snus use. Leukoplakia is the most common type of pre-malignant lesion. Research reveals contradictory information about the risk of oral cancer for snus users [25]. Due to ONPs being similar to snus, there is a possibility that they could cause similar changes in the oral mucosa tissues; however, there are not enough studies to support this hypothesis, and further observations are needed.

Systemic effects of nicotine. The liver plays a crucial role in absorbing and processing nicotine once it has been absorbed into the body. Typically, only a small percentage (around 5–10%) of nicotine remains unchanged and is excreted through the kidneys. However, when the pH in urine is high, some nicotine can be reabsorbed from the bladder. It is worth noting that within living organisms, nicotine can undergo a process called nitrosation, leading to the formation of highly carcinogenic compounds, e.g. N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). This risk of nitrosation is heightened in the presence of oral inflammation [26]. Nicotine affects the body in three main ways: it affects ganglionic transmission, interacts with nicotinic acetylcholine receptors (nAChRs) on chromaffin cells through catecholamines, and stimulates the central nervous system (CNS) via nAChRs.

Nicotine is generally accepted as one of the most addictive substances, its addictiveness often compared to that of cocaine or heroin. The mechanism of action involves interaction with nicotinic acetylcholine receptors and the stimulation of dopaminergic transmission. Additionally, nicotine triggers an elevation in oxidative stress, neuronal apoptosis, DNA damage, and an increase in reactive oxygen species and lipid peroxide levels. These actions on nicotinic receptors have a variety of immediate and long-term effects on organ systems, cell growth, and cell death throughout the body.

The direct application of nicotine in humans can cause irritation in the mouth and throat, a burning sensation, increased saliva production, nausea, stomach discomfort, vomiting, and diarrhea. It also causes an increase in heart rate and blood pressure. Furthermore, nicotine induces an upsurge in plasma free fatty acids, raises blood sugar levels (hyperglycaemia), and elevates catecholamine levels in the bloodstream [26].

Table 1. Harmful effects of nicotine (26)

METABOLISM	Promotes lipolysis, leading to weight loss, impacting insulin sensitivity, and increasing the likelihood of developing metabolic syndrome and diabetes
CARCINOGENESIS	Activation of nicotinic acetylcholine receptors (nAChRs) on cells is crucial for the onset and development of cancer. Nicotine can act as a tumor promoter in the lung, gastrointestinal system, breast, and pancreas.
CARDIOVASCULAR SYSTEM	reduces blood flow in cutaneous and coronary vessels; and increases blood flow in the skeletal muscles, increases the risk of peripheral arterial disorders
RESPIRATORY SYSTEM	Bronchoconstriction and apnea increase tracheal tension, leading to various respiratory disorders.
GASTROINTESTINAL SYSTEM	Nicotine use has been associated with Gastro Esophageal Reflux Disorder (GERD) and peptic ulcer disease (PUD)
IMMUNOLOGICAL SYSTEM	Multi-mechanism immunosuppressive effect
OCULAR SYSTEM	Relationship between nicotine and glucose metabolism exaggerating diabetes might cause accelerated cataract formation
RENAL SYSTEM	Increased glomerular inflammation, acute glomerulonephritis and ureteral obstruction
REPRODUCTIVE SYSTEM	Male: loss of penile erections and erectile dysfunction, decrease testosterone levels Female: chronic anovulation and irregular menstrual cycles, effects on the endocrine system

Effects of nicotine on periodontal tissue. Gingivitis, periodontitis and peri-implantitis are different types of periodontal disease which are linked to several systemic disorders, and are the main reason for tooth loss. The development of periodontal disease is caused by an imbalance between the host's defensive system and the pathogenic bacteria. Epidemiological studies have unequivocally identified smoking as a significant risk factor for periodontal disease [23].

The immune system, microbiota, and the periodontium's self-repair capabilities are all impacted by smoking. It has been proposed that smoking may cause an alteration in the subgingival biofilm composition [27]. Smoking has also been linked to a delay in the recruitment and migration of neutrophils into periodontal tissues, which compromises the acute immune response [27]. This would raise the threshold of aggression that the periodontal tissue has to reach in order to initiate the inflammatory cascade. In addition, it has been proposed that smoke could alter the neutrophil activity balance to a more destructive behaviour. Specifically, an elevation in the ratio between the receptor activator of nuclear factor-ligand (RANKL) and its inhibitor osteoprotegerin (OPG) leads to an increase in bone resorption due to higher interleukin (IL)-1 and IL-6 levels. Moreover, nicotine induces an up-regulation in the production of other cytokines, such as CXCL8, IL-10, and IFN-c (interferon-c), as well as prostaglandin E2 (PGE2), while simultaneously down-regulating matrix metalloproteinases – MMP2.

Studies conducted *in vitro* reveal that nicotine exposure significantly increase the expression of the nicotinic acetylcholine receptor (nAChR) [28]. Diminished expression of nAChR curbed the activity of periodontal ligament (PDL)

fibroblasts and decreased the viability of stem cells, while amplifying the production of cellular reactive oxygen species (ROS). The escalated ROS levels consequently initiated a cascade of signalling events, including the activation of pathways, such as ERK (extracellular signal-regulated kinases), JNK (c-Jun N-terminal kinases), caspase-3, and caspase-9, ultimately resulting in DNA fragmentation and cellular demise [23, 29, 30].

In the context of the cell co-culture model, nicotine exacerbates the progression of periodontitis by stimulating periodontal ligament (PDL) cells to secrete CXCL12 which, in turn, attracts CD4+ T cells. Consequently, this cascade leads to an increased expression of MMP-1, MMP-3, IL-1, IL-6, IL-17, and IL-21 [23]. Smokers showed an increase in the levels of alpha-2-macroglobulin and -1-antitrypsin, both of which are protease inhibitors, and a decrease in elastase, MMP-8, and MMP-9, which are proteases. This could potentially hinder the healing of the periodontal tissues by increasing collagenolytic activity and decreasing the fraction of blood vessels in the gums [27]. By causing local irritation and boosting blood pressure, nicotine consumption overrides neurological and hormonal vasoconstriction, thus leading to a higher rate of blood flow to the oral mucosa. Chronic tobacco smokers exhibit greater gingival microvascular density, primarily due to increased capillary recruitment. Nevertheless, these microcirculatory units feature higher curves and a diminished diameter. For individuals with periodontal disease who are non-smokers, there is noticeable gingival inflammation and angiogenesis; however, this is greatly reduced in chronic smokers, likely due to immunosuppression and increased oxidative stress. No matter the form of nicotine consumption, exposure to it leads to long-term impairment of the microvasculature, increasing the chances of complications from the disease's natural progression or other treatments [31]. It is highly probable that the predominant association between ONPs and periodontal diseases can be attributed to the deleterious impact of nicotine on the oral and periodontal tissues.

MicroRNAs (miRNAs) have the capacity to modulate gene expression and biological activities, playing crucial roles in the pathophysiology of periodontitis. They are implicated in the regulation of various biological processes, molecular functions, cellular components, and signalling pathways, including NF-κB, epithelial-mesenchymal signalling, and SMAD signalling. An *in vitro* study demonstrated that human periodontal ligament cells (PDLCs) exposed to nicotine had up- and down-regulations of miRNAs [32]. It is implied that oral nicotine pouches, along with all other nicotine-containing products, may have the potential of initiating or exacerbating periodontal disease by means of miRNA-controlled processes, which may eventually lead to oral submucous fibrosis.

Nicotine found in oral nicotine pouches has been linked to alveolar bone loss, a defining feature of periodontal disease. Nicotine absorbed locally attracts inflammatory cells into the periodontal tissues, facilitating cellular interactions and triggering the production of factors that promote osteoclast formation, such as RANKL, RANK, TNF, and IL-1β. This, in turn, contributes to the induction of osteoclasts and exacerbates bone degradation [23].

Potential molecular targets and toxic effects resulting from ONPs exposure have been demonstrated, initiating multiple signalling pathways, e.g. AKT (protein kinase B) and NF-kappaB, potentially resulting in apoptosis and epithelial mesenchymal transition (EMT). Following the use of ONPs on human gingival epithelial cells (HGEPp), elevated levels of lactate dehydrogenase (LDH), ROS, and inflammatory cytokines (TNF, IL-6, and CXCL8) can be anticipated [33].

Risk other than nicotine. In a recent study, a comparison of the harmfulness of ONPs to tobacco-containing products was performed [20][34] which described the content and release of nicotine and other dangerous substances associated with tobacco. The study demonstrated that a nicotine pouch contains, on average, almost 50 mg of nicotine, as well as small amounts of tobacco carcinogens, such as N-nitrosonornicotine (NNN) or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)[34]. Nevertheless, more detailed knowledge of their hazardous components is needed in order to carry out a thorough risk assessment of these products. These substances, which have been neglected in previous investigations, are not uncommon in tobacco products.

Screening studies of 48 different nicotine-containing pouches were conducted using gas chromatography coupled with mass spectrometry, following both acidic and alkaline liquid-liquid extraction. The primary compounds detected included: sweeteners, flavouring agents, humectants, fillers, and acidity regulators. The manufacturer's official product formulations did not include all of the substances identified in the study. The toxicity of the discovered substances was evaluated through contrasting the outcomes of these analyses to European and international standards of chemical and food safety. When pouches are used with moderate frequency, the daily intake limitations specified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) may be exceeded. The European CLP Regulation (Classification, Labelling and Packaging) was applied to classify eight dangerous chemicals. The EFSA denied the use of 13 chemicals, including toxins like myosmine and ledol, used as flavouring agents in food. The International Agency for Research on Cancer (IARC) has suggested that three chemicals – methyl eugenol, benzophenome, and β-myrcene – could be responsible for causing human cancer [15].

Detecting a large number of toxic substances does not necessarily result in 'product use risk'. Incorporating 'exposure' is essential. Hence, it is critical to examine toxicological limits and safety thresholds, as the result is contingent on daily intake. Regulatory bodies and institutions like the EFSA and JECFA have established Acceptable Daily Intake (ADI) values for numerous substances. Ensuring that the daily consumption of these substances remains below their ADI thresholds is deemed safe, accounting for all potential sources of intake. A user weighing 70 kg was estimated to use an average of five nicotine pouches per day, and up to 20 pouches per day in the most extreme circumstances. Therefore, it is possible that these chemicals might have negative consequences [15].

Menthol flavouring encourages the diffusion of dangerous substances, such as nitrosonornicotine (NNN) and nicotine, through the buccal and floor-of-mouth mucosa, raising the possibility of oral soft tissue damage [23]. It is probable that nicotine, which is present in some items at extremely high levels, is the origin of the acute toxicity experienced orally. The possibility exists that these components (carvone, linalool, limonene, geraniol, isoeugenol, citral) may act as

Table 2. Liquid-liquid extraction and GC/MS analysis of 48 nicotine pouches yielded two compounds as examples of what can be found in nicotine pouches (15)

classified as possibly carcinogenic to humans (cat. 2B) by IARC	• methyl eugenol (induces liver tumors in rodents) • benzo- phenome (induces kidney and liver cancer as well as histiocytic sarcomas in rodents) • β-myrcene (renal and liver tumors in rats and mice) all three substances were detected in less than four products
not on the list list of authorized food flavorings by the EU	• tris(2-butoxyethyl)phosphate (possible hepatotoxic and carcinogenic properties) • isomenthyl acetate, • cis-β-farnesene, • myosmine (tobacco alkaloid, impurity of nicotine, degradation product of nicotine, reagent used in nicotine synthesis) • ledol (effects on the central nervous system, thereby causing dizziness, nausea, and vomiting, among other symptoms) • saccharin, • pulegone, • isomenthol, • neoisomenthol, • humulene, • cis-carvon oxide, • estragole, • methyl eugenol.
Classifications according to CLP	H317 skin sensitizing properties, allergic reaction • carvone, • linalool, * • limonene, * • geraniol, * • isoeugenol, * • citral * *listed as fragrance allergens according to Annex III of the Cosmetics Regulation H361d suspected of damaging the unborn child • Salicylic acid H372 causes damage to organs through prolonged or repeated exposure • Benzoic acid (inhalation - irrelevant in this evaluation, lungs)

local sensitizers in epithelial cells, or be systemically absorbed due to lengthy contact with the oral mucosa [15].

Furthermore, a research group headed by Shaikh conducted a detailed study in order to examine the cytotoxicity of smokeless tobacco products in accordance with their brand, flavour, and tobacco content [33, 35]. Their research indicated that ONPs could be potentially damaging to oral epithelial cells due to the release of ROS and inflammatory cytokines like TNF, IL-6, and CXCL8, which have the potential to cause various oral and lung problems. The highest cytotoxicity was observed when using fruit extracts. Four hours after treatment, the ROS generation was elevated by ONPs with fruit and tobacco flavours, but not menthol. The authors of the study believe that ONPs are not less toxic than snus, and they emphasize that toxicity levels differ depending on the flavour. After testing a few selected flavours, it was found that menthol-flavoured ONPs (wintergreen and cool cider) significantly increased TNF release; fruit flavours (citrus and americana) significantly raised IL-6 release and tobacco (original, smooth) substantially increased CXCL8 release [35]. Therefore, localized contact with toxic substances may lead to the initiation of inflammatory responses in the oral mucosa. In another study, five various nicotine pouches and the benchmark snus were tested for toxicity *in vitro* using human gingival fibroblasts (HGF-1). Elevated gene expression of IL-6 and heme oxygenase 1 (HMOX1), as well as heightened production of ROS.

Importantly, toxicity was not directly linked to nicotine concentration or osmolarity [36]. These results suggest that chronic use of these products may lead to adverse effects, such as local mucosal changes in the buccal area. In conclusion, the flavourings found in nicotine pouches may play a significant role in the overall toxicity of these products containing nicotine. Both nicotine-independent and nicotine-dependent effects can be observed. There is also the probability of a synergistic interaction between nicotine and other components, such as flavourings, on cells [36].

Examining the microbiome of individuals exclusively using ONPs is complex due to their history of cigarette smoking or current engagement in the habit. Consequently, the bacterial species inhabiting the oral cavity are expected to exhibit similarities to those found in cigarette smokers. To address this, animal model studies were conducted which revealed notable differences in bacterial composition. Animal studies also revealed that snus exposure led to an increase in the number and types of periodontitis-causing bacteria, including *Actinomyces*, *Streptococcus*, and *Staphylococcus* [37]. Use of flavoured nicotine products could lead to microbial dysbiosis of the oral cavity and periodontium [23].

Table 3. Influence of Nicotine Pouches on the periodontium

EFFECTS OF NICOTINE	upregulation of inflamatoty cytokines like IL-1, IL-8, IL-6, IL-10, downregulating MMP2, , alveolar bone loss, dysbiosis, apoptosis, epithelial mesenchymal transition elevated levels of lactate dehydrogenase (LDH), reactive oxygen species (ROS), oxidative stress, dysregulation of miRNAs, dysfunction in microvasculature
IMPACTS OF NON-NICOTINE COMPOUNDS	irritation or mechanical trauma, allergic reaction, cytotoxicity, possibly carcinogenic

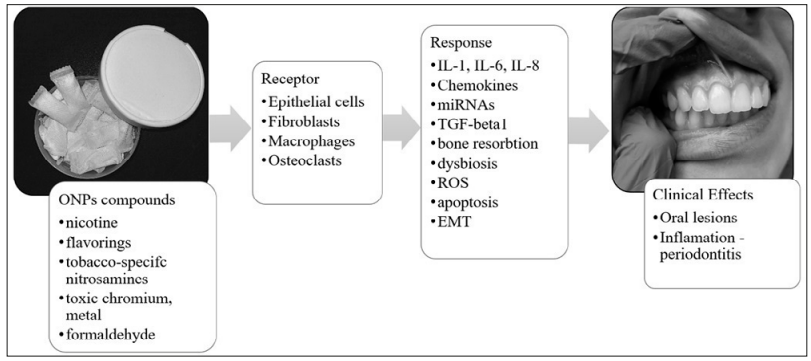

Figure 4. Influence of nicotine pouches. TGF-β1 (transforming growth factor beta1)

CONCLUSIONS

Monitoring ONP use is vital for public health due to its recent emergence and limited understanding of its effects, despite perceptions of being less harmful, their overall health impact, especially on oral health, remains uncertain. While ONPs are seen as a 'lesser evil', caution is warranted due to potential overlooked health risks. It is important to stress that the long-term use of any substances, particularly those absorbed via the oral mucosa and used chronically, is especially dangerous and poses potential risks that cannot be fully assessed after only a few years of study. Medical practitioners must prioritize patient well-being over ONP use, emphasizing cessation over harmful options.

Research gaps exist regarding the impact of ONPs on oral health, compounded by limited exclusive users, necessitating further study, particularly on synthetic compounds and usage patterns. Monitoring potential oral cavity harmful effects and gathering clinical data are crucial for validating ONPs as nicotine replacement therapy. It is important to highlight the importance of conducting further research on the impact of these new products on public health, and the potential future consequences that may arise.

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