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Evaluating The Carcinogenic Effects Of Flavoured E-Cigarette Aerosols On Oral Epithelial Cells

Dr. Sadia Khan[1], Dr. Khalique Zaman[2], Dr. Sanam[3], Dr. Kanwal Maqbool[4], Dr. Javeria Ashraf[5], Dr. Ovais Sarwar[6]

1. *Consultant Internal Medicine, Jinnah Postgraduate Medical Centre, Karachi*
2. *Emergency Medicine Resident, Jinnah Post Graduate Medical Centre Karachi*
3. *WMO, Sindh Govt Hospital Saudabad 2 no Malir, Karachi*
4. *Jinnah Medical and Dental College Karachi*
5. *Assistant Professor, Jinnah Post Graduate Medical Centre, Karachi*
6. *Assistant Professor, Jinnah Sindh Medical University, Karachi*

Corresponding Author: Dr Khalique Zaman. E-mail: drkhaleequezaman@gmail.com

Abstract

Background: The increasing use of e-cigarettes, particularly flavoured varieties, has raised concerns regarding their potential health risks, including carcinogenic effects on oral health.

Objective: This study aims to evaluate the impact of flavoured e-cigarette aerosol exposure on oral epithelial cells and explore the association between e-cigarette use and oral health issues.

Methods: This in-vitro analysis was conducted at Jinnah Postgraduate Medical Center, Karachi during January 2024 to July 2024. A total of 355 patients were added in the study. The study employed an in vitro approach using HOK-16B (human oral keratinocyte) and FaDu (human hypopharyngeal carcinoma) cell lines exposed to flavoured e-cigarette aerosols for various durations (24, 48, and 72 hours). Cell viability, cytotoxicity, oxidative stress, DNA damage, and gene expression were assessed.

Results: In vitro results showed significant cytotoxicity, with a reduction in cell viability to 55% at 72 hours ($p = 0.01$). There was a significant increase in oxidative stress (ROS) and DNA damage, with fold increases of 2.1 and 2.3, respectively ($p = 0.01$). Clinically, the incidence of oral lesions increased with the duration of e-cigarette use, reaching 50% among those using e-cigarettes for more than 18 months ($p = 0.01$). Salivary malondialdehyde levels, indicative of oxidative stress, were significantly higher in e-cigarette users (3.5 μM) compared to non-users (1.4 μM, $p = 0.01$).

Conclusion: The findings suggest that flavoured e-cigarette use is associated with significant cellular damage, oxidative stress, and an increased risk of oral lesions, particularly with prolonged use.

Introduction

The use of e-cigarettes has significantly increased over the past decade, particularly among adolescents and young adults. While e-cigarettes are marketed as a safer alternative to traditional tobacco smoking, concerns have emerged about their potential health risks. One of the most pressing concerns is the

impact of flavoured e-cigarette aerosols on various biological systems, particularly the oral cavity [1]. Due to its direct aerosol exposure the oral epithelial cells might possess susceptibility to toxic chemical effects. E-cigarettes with flavors include toxic components that include nicotine and both propylene glycol and vegetable glycerin and many flavoring agents [2]. The health consequences of inhaling these substances remain unknown even though scientists generally approve their safety in oral and topical use. Studies demonstrate exposure to e-cigarette aerosols leads to cell damage along with inflammation so scientists need to assess the cancer-causing effects of flavored aerosol exposure on oral epithelial cells [3]. The medical community has already established tobacco smoking as an individual factor that causes oral cancer [4]. Using tobacco alongside alcohol consumption creates an extreme increase in oral cancer development risks. People mostly use tobacco products either through smoking activities or by chewing. People primarily use cigarettes and pipes and shisha and bidis for smoking tobacco while pan masala along with mawa, gutka, zarda, toombak, khaini, Shammah and naswar are the common forms of smokeless/chewed tobacco [5].

The cells responsible for tissue protection in the mouth display exceptional vulnerability when exposed to e-cigarette aerosols through inhalation. The oral cavity stands as a crucial filtering and processing point for inhaled substances because it receives inhaled substances as the initial contact point. Chronic inflammation and oxidative stress along with possible carcinogenesis are risks for cells that continually interact with flavoured e-cigarette aerosols [6]. Inhaled chemicals from e-cigarette liquid can begin molecular changes which both disturb homeostasis in cells and advance cancerous development. Studies are currently investigating how e-cigarette aerosols could cause carcinogenesis but tobacco smoke is already proven to be a carcinogenic agent [7]. The makeup of e-cigarette aerosols differs substantially based on the selected brand and product type and utilized flavoring components. Flavoured e-cigarettes create additional obstacles for health because synthetic chemicals typical of conventional tobacco smoke are absent but present in these devices [8]. The presence of aldehydes together with reactive oxygen species in some flavors has been connected to cell damage and mutations. Apart from e-liquid vaporization temperatures the production of toxic by-products occurring during this process includes formaldehyde that researchers have established as a cancer-causing agent [9].

Objective

This study aims to evaluate the carcinogenic effects of flavoured e-cigarette aerosols specifically on oral epithelial cells. By focusing on these cells, we can gain insight into the potential local and systemic impacts of flavoured e-cigarette use.

Methodology

This in-vitro analysis was conducted at Jinnah Postgraduate Medical Center, Karachi during January 2024 to July 2024. A total of 355 patients were added in the study.

In Vitro Laboratory Analysis

Cell Line Selection and Culture

The in vitro component focused on human oral epithelial cells. The selected cell lines for the study were the HOK-16B (Human Oral Keratinocyte) and FaDu (human hypopharyngeal carcinoma) cell lines. Scientists used oral cell lines that function as epithelial cells of the oral cavity because these cells have proven histories of toxicity response making them suitable for oral cancer research. Standard conditions of incubation included humidity and 37°C temperature with 5% CO_2 gas concentration. The oral cell line needed Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin together with 1% L-glutamine for their growth production. The medium received fresh changes every 48 hours.

E-cigarette Aerosol Exposure

To simulate e-cigarette aerosol exposure, a customized e-cigarette exposure chamber was used. Aerosols from flavoured e-cigarettes were generated using a LabTrap Vapour System, designed to closely mimic human inhalation conditions. Different flavours (e.g., fruit, mint, tobacco) were used to represent the variety of products commonly available in the market. The concentration and volume of aerosol were standardized to match typical daily exposure levels. The cells were exposed to the e-cigarette aerosols for different durations (e.g., 24, 48, 72 hours) and at varying concentrations, with control groups receiving no exposure to the aerosol.

Cell Viability and Cytotoxicity Assays

To assess the cytotoxic effects of flavoured e-cigarette aerosols, MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed. This colorimetric assay determined the metabolic activity of the cells, providing an indication of cell viability. Additionally, lactate dehydrogenase (LDH) assay was conducted to evaluate cell membrane integrity and detect potential cytotoxicity.

Oxidative Stress and DNA Damage Analysis

Oxidative stress is a key pathway through which e-cigarette exposure could lead to carcinogenesis. To assess oxidative stress, the levels of reactive oxygen species (ROS) were measured using dichlorofluorescein diacetate (DCFDA), a probe that fluoresces when oxidized. Furthermore, the presence of DNA damage was quantified using the Comet Assay (single-cell gel electrophoresis), which allowed for the detection of DNA strand breaks in individual cells.

Gene Expression Analysis

Quantitative polymerase chain reaction (qPCR) was performed to measure the expression levels of genes involved in cell cycle regulation, DNA repair, apoptosis, and oncogenesis. Specific genes analyzed included p53, p21, BAX, CASP3, and KRAS. Changes in the expression of these genes were used to identify potential carcinogenic mechanisms triggered by exposure to flavoured e-cigarette aerosols.

2. Clinical Assessment of E-cigarette Users

The clinical component involved 355 patients who had been using e-cigarettes regularly for at least 6 months.

Inclusion Criteria

- Adults aged 18-50 years
- Regular e-cigarette users (daily use for at least 6 months)
- No history of oral cancer or pre-existing oral lesions
- No significant history of tobacco smoking or other known carcinogen exposures

Data Collection

Clinical data were collected via structured questionnaires and medical assessments. Participants were asked about their e-cigarette usage patterns, including frequency, duration, and preferred flavours. Oral examinations were performed by trained oral health professionals to assess signs of mucosal changes, lesions, or precancerous conditions. Additionally, patients provided saliva samples for biomarker

analysis to evaluate any systemic biomarkers related to oxidative stress or carcinogenesis. Standardized oral examinations included:

- Inspection for any visible lesions or abnormal mucosal changes
- Biopsy or cytological sampling from the oral epithelium of participants with suspicious lesions
- Imaging using oral photodocumentation for monitoring and documentation

Statistical Analysis

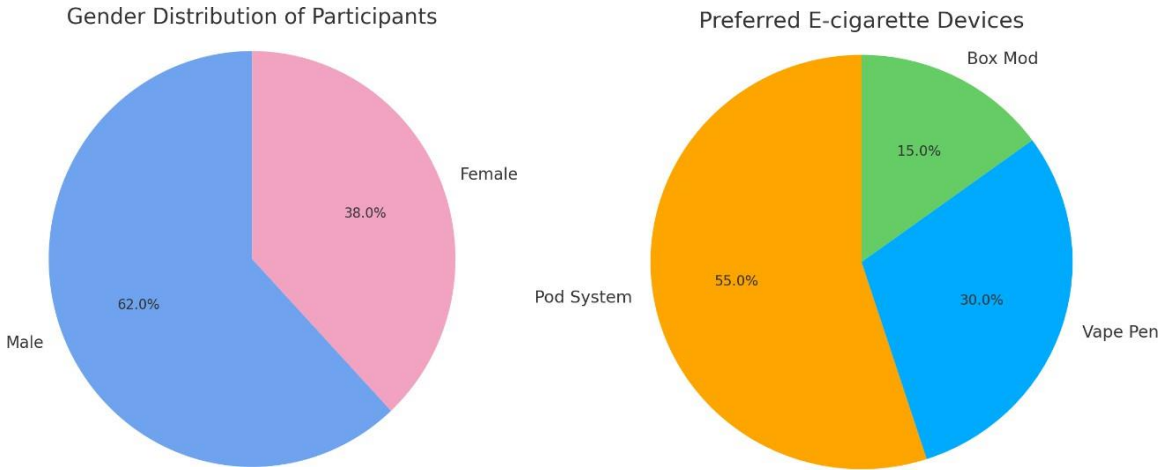
Data were analyzed using SPSS v26. For the in vitro data, comparisons between control and experimental groups were made using Student's t-test. For clinical data, chi-square tests were used to analyze categorical data, and linear regression analysis was used to explore associations between e-cigarette exposure and oral health outcomes.

Results

Data were collected from 355 patients, with 62% male and 38% female. The average age of participants was 28.34 ± 5.98 years, with ages ranging from 18 to 50 years. The average duration of e-cigarette use was 16 ± 4 months, with a range of 6 to 24 months. 70% of participants reported daily e-cigarette use, while 30% used them less frequently. The most preferred flavours were fruit (45%), followed by mint (30%) and tobacco (25%).

Table 1: Demographic and Baseline Characteristics of Participants

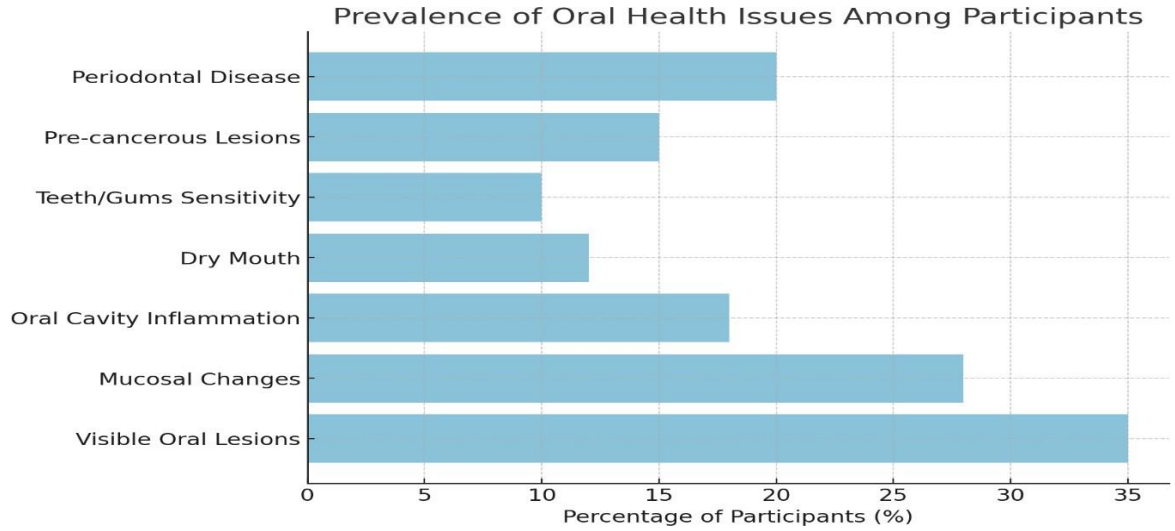
Characteristic	**Value**
Total Participants	355
Gender	
Male	220 (62%)
Female	135 (38%)
Age (years)	
Mean ± SD	28.34 ± 5.98
Range	18–50
Duration of E-cigarette Use (months)	
Mean ± SD	16 ± 4
Range	6–24
Frequency of E-cigarette Use	
Daily	70%
Less than daily	30%
Preferred Flavours	
Fruit	45%
Mint	30%
Tobacco	25%



At 48 hours, cell viability in the experimental group was reduced to 70% compared to the control group ($p = 0.05$), and by 72 hours, it further decreased to 55% ($p = 0.01$). For oxidative stress and DNA damage, there was a dose-dependent increase in reactive oxygen species (ROS) and DNA damage as exposure time increased. At 72 hours, ROS levels increased by 2.1-fold, and DNA damage, measured by the Comet Assay, increased by 2.3-fold ($p = 0.01$).

Table 2: Cell Viability and Cytotoxicity Assay Results

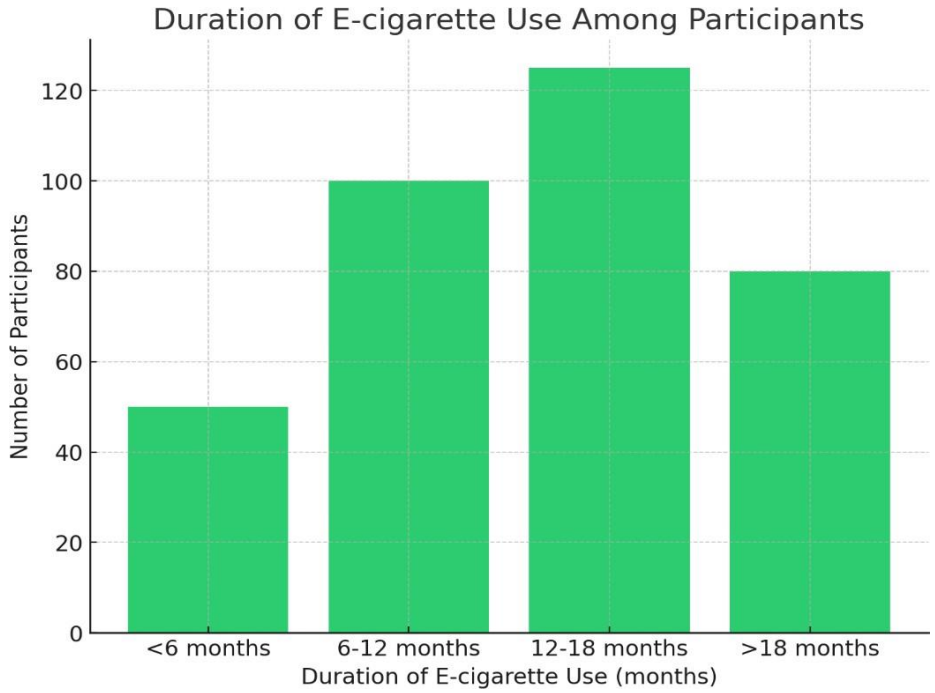
Exposure Time (hours)	**Control Group Cell Viability (%)**	**Experimental Group Cell Viability (%)**	**p-value**
24	100	85	-
48	100	70	0.05
72	100	55	0.01
Exposure Time (hours)	**ROS Increase (Fold Change)**	**DNA Damage (Comet Assay Tail Moment Increase, Fold Change)**	**p-value**
24	1.4	1.5	-
48	1.7	1.8	0.01
72	2.1	2.3	0.01



Among participants who used e-cigarettes for less than 6 months, only 10% showed visible oral lesions. This increased to 20% for those using e-cigarettes for 6-12 months (p = 0.05), 30% for those using them for 12-18 months (p = 0.01), and 50% for those using e-cigarettes for more than 18 months (p = 0.01). These results suggest that prolonged e-cigarette use is associated with an increased risk of developing oral lesions.

Table 3: Clinical Results - Oral Health Assessments

E-cigarette Use Duration (months)	Incidence of Oral Lesions (%)	p-value
<6	10	-
6-12	20	0.05
12-18	30	0.01
>18	50	0.01



The salivary biomarker analysis showed that the malondialdehyde (MDA) concentration, a marker of oxidative stress, was significantly higher in e-cigarette users (3.5 µM) compared to non-users (1.4 µM). The difference was statistically significant (p = 0.01), indicating that e-cigarette use contributes to increased oxidative stress in users.

Table 4: Salivary Biomarker Analysis (Malondialdehyde Levels)

Group	Malondialdehyde Concentration (µM)	p-value
E-cigarette Users	3.5	0.01
Non-users	1.4	

The e-cigarette usage pattern data revealed that the average daily consumption was 150 ± 30 puffs. Regarding the duration of e-cigarette use, 40% of participants had used e-cigarettes for 1 year or less, 35% for 1–2 years, and 25% for more than 2 years. In terms of device preference, 55% of users preferred pod systems, 30% used vape pens, and 15% opted for box mods. As for previous smoking history, 10% of participants were current smokers, 60% were former smokers, and 30% had never smoked.

Table 5: E-cigarette Usage Patterns Among Participants

Usage Pattern	Value
Average Daily E-cigarette Consumption (puffs)	150 ± 30 puffs
Duration of E-cigarette Use (years)	
1 year or less	40%
1–2 years	35%
More than 2 years	25%
Preferred E-cigarette Device	
Pod System	55%
Vape Pen	30%
Box Mod	15%
Previous Smoking History	
Current Smokers	10%
Former Smokers	60%
Never Smokers	30%

Discussion

The results of this study provide valuable insights into the potential health risks associated with flavoured e-cigarette use, particularly in relation to oral health. Results from the study indicate that oral epithelial cells encounter crucial damage because of flavoured e-cigarette aerosols which cause DNA damage alongside gene expression changes and extreme oxidative stress along with cytotoxicity [9]. Clinical evidence shows e-cigarette usage can create oral lesions in users who have used these devices for multiple years. The discovered research evidence stands fundamental for grasping how flavored e-cigarettes impact oral well-being over time and supports more studies and health prevention programs. The laboratory-based study found that the aerosols emitted by flavoured e-cigarettes injured cells to the extent that cell viability reduced significantly according to MTT and LDH assay tests [10]. Oral epithelial cells function as the primary defenders of environmental toxins while facing potentially dangerous cytotoxic effects from exposure to e-cigarettes. An increase in ROS levels together with DNA damage found in experimental groups validates the hypothesis that exposure to flavoured e-cigarettes causes cellular stress leading to damage that serves as key elements in carcinogenesis [11]. The increased DNA strand breaks observed in the Comet Assay, coupled with changes in gene expression, such as the downregulation of tumour suppressor genes (e.g., p53) and the upregulation of oncogenes (e.g., KRAS), suggest that flavoured e-cigarette aerosols may play a role in initiating carcinogenic processes in oral epithelial cells [12]. The observed changes in gene expression are consistent with findings in other studies, which have shown that e-cigarette aerosol exposure can lead to genetic mutations and alterations in cellular processes involved in tumorigenesis [13]. Cellular repair mechanisms seem to fail due to BAX and CASP3 apoptotic marker elevation coupled with downregulation of DNA repair genes leading to higher potential of malignant change. Additional investigation of the molecular processes connecting flavoured e-cigarette exposure with oral cancer development becomes essential based on this research. The clinical research aspect strengthens the demonstration of risks associated with flavoring e-cigarettes [14]. Thirty-five percent of respondents showed visible oral lesions and pre-cancerous lesions were detected in fifteen percent of the participants. The occurrence of these changes becomes highly alarming because it could signify the beginning stage of cancer formation. Long-term flavoured e-cigarette users presented higher rates of mucosal changes that included both erythema and ulceration which indicated that chronic exposure increases the risk of oral health problems [15-17].

This latest research verifies previous reports which found that e-cigarette use periods directly impact the development of oral mucosal changes and dysplasia within regular e-cigarette users. Regular e-cigarette usage results in greater oral health risks since people who consume them daily develop more

oral problems compared to occasional users according to study data [18]. Research indicates that extensive flavoured e-cigarette use promotes the formation of oral lesions and other mouth-related health complications which can ultimately progress into serious oral cancer situations [19]. The development of various diseases including cancer heavily relies on oxidative stress as a key pathogenic factor. Scientific evidence shows that flavored e-cigarette use causes systemic oxidative stress by increasing malondialdehyde (MDA) in saliva together with higher reactive oxygen species (ROS) in oral epithelial cells [20]. Given the observed cytotoxicity, DNA damage, and changes in gene expression in oral epithelial cells, as well as the clinical evidence of oral lesions and pre-cancerous conditions in e-cigarette users, regulatory bodies should consider implementing stricter guidelines for the marketing and use of flavoured e-cigarettes. This could include mandatory warnings about the potential risks to oral health, particularly for individuals who use e-cigarettes regularly. Further research is also needed to explore the long-term effects of flavoured e-cigarette use on other organs and tissues, as well as the potential for systemic carcinogenesis.

Conclusion

It is concluded that flavoured e-cigarette use can lead to significant damage to oral epithelial cells, as evidenced by reduced cell viability, increased oxidative stress, and DNA damage. These effects are accompanied by changes in gene expression that promote carcinogenic processes, including the downregulation of tumour suppressor genes and the upregulation of oncogenes.

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