

EFFECTS OF ELECTRONIC CIGARETTES AND NICOTINE ON THE VENTRAL SURFACE OF RAT TONGUE AND THE POSSIBLE ROLE OF A COMBINATION OF VITAMIN C AND E.

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ABSTRACT:

OBJECTIVE: The aim of this paper is to study structural changes caused by nicotine and electronic cigarette refill liquid in ventral surface of rat tongue and the possible effects of supplementation with vitamins C and E combined.

DESIGN: 40 adult albino rats of male gender, weighing (200–300 gms) were allocated into 5 groups; control, electronic cigarettes (EC), electronic cigarettes group supplemented with vitamin C and E combination (EC plus V), nicotine (N) group and nicotine group given vitamin C and E combination (N plus V). Specimens were prepared for histological examination by light microscopy and histomorphometric analysis of total surface areas. One-way ANalysis of VAriance (ANOVA) test. When F ratio of ANOVA was significant Levene test of homogeneity of variances was used, and if significant Brown-Forsythe Robust test was adopted. Games-Howell was used in Post-hoc multiple comparisons, with a 5% significance level.

RESULTS: Detrimental degenerative changes were observed in nicotine and electronic cigarettes groups. Preservation of the normal cellular organization was seen in nicotine and electronic cigarettes with supplementation of vitamin C and E combined.

CONCLUSIONS: Vitamin C and E combination can preserve normal features of ventral surface of rat tongue epithelium subjected to nicotine or electronic cigarettes.

KEYWORDS: electronic cigarettes, nicotine, vitamin C, vitamin E, ventral surface, tongue.

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INTRODUCTION:

The oral cavity is lined by a moist coverage, named oral mucosa. It is considered the principal barrier between oral environment and deeper tissues. Thus, protection is one of its primary functions along with several others. The oral mucosa of tongue ventral surface consists of 2 main components; an outer covering epithelium and underlying supporting connective tissue. The oral epithelium is a stratified squamous epithelium consisting of cells tightly attached to each other and organized as distinct layers. (1)

In 2015, the WHO estimated that the percentage of worldwide tobacco smoking is 20.2%. Even though tobacco products consumption has declined, an increase in the incidence of using electronic cigarettes occurred among teenagers. 27% of american high-school students reported current use of tobacco products. (2,3)

According to the Centre for Disease Control (CDC) and Prevention, smokers are categorized into current smokers, non-smokers, and

former smokers. A current smoker is defined as an individual who smoked a minimum of 100 cigarettes in their lifetime, and who still currently smokes. (4) Moreover, smokers can be classified, according to the number of cigarette rods consumed daily, into light smokers (smoking less than 5 rods), moderate smokers (6–16 rods), and heavier smokers (smoking more than 16 rods). (5,6)

Smoking can cause inflammatory responses related to oral mucosa due to heat emission, smoke, and other cigarettes' constituents. These elements are considered as risk factors of oral mucosa inflammation, recruitment of inflammatory cells and development of cancer orally. (7) Nicotine is a main psychoactive and addictive component of cigarette smoke. (2,8,9)

Electronic cigarettes' (ECs) popularity escalated as they are regarded as a safer alternative to traditional cigarettes. They are advertised to help users decrease or quit smoking habit. Their emergence offered smokers an alternative method to attain nicotine. (10,11) However, there are

several adverse effects for ECs such as; the spike in occurrence of oral cavity lesions in contrast to non-users.(11)

Smoking is a preventable reason of premature death and is a risk factor for many diseases as, cardiovascular, and respiratory diseases (12,13) Smokers' nutrients intakes are lower. Moreover, they may have elevated need for certain nutrients due to high metabolic demand. The Dietary Reference Intake committee recommends 90 mg/day of vitamin C intake, however, smokers should have additional 35 mg/day to recompense increased turnover of vitamin C and high oxidative stress induced(13,14)

Vitamin C is a water-soluble antioxidant, which is concerned mainly with the protection of the aqueous parts of cells from oxidative damage and stress. (15,16) The main route of administration of vitamin C is orally from food or supplements. Healthy individuals need daily 0.1–0.2 gm of vitamin C. (17)

Vitamin E is an effective lipophilic antioxidant that acts by scavenging free radicals. Therefore, it protects the lipid components of cell membranes. (15,18,19) Furthermore, vitamin E plays a role in inhibition of cancer cell growth along with cessation of protein synthesis in cancer cells. Therefore, it reduces the risk of developing oral cancer. (20) This study aims to evaluate the structural differences caused by nicotine and electronic cigarette refill liquid in ventral surface of rat tongue. The null hypothesis is no difference will be detected in ventral surface of smokers' tongue histological structure upon supplementation with Vitamins C and E combined.

MATERIALS AND METHODS:

Study subjects:

40 adult male albino rats, with body weights ranging (200–250 gms) were kept in experimental animal house located in Faculty of Medicine, Alexandria University. Sample size calculations were done in department of Biomedical Informatics and Medical Statistics, Medical Research Institute. To fulfill 80% power of the study and 95% confidence level, the minimum needed sample size was 8 rats in each group (groups = 5) (sample size= 40 rats) based on previous literature reviews.(21,22) Animals were kept in wire mesh bottom cages, at room temperature. They were supplied with normal diet and drinking tap water. Research Ethics Committee, Faculty of Dentistry, Alexandria University approved the current study

Study groups:

Animals were allocated into 5 groups randomly:

Control group: 8 rats injected daily with saline intraperitoneally for 5 weeks.

Electronic cigarette (EC) group: 8 rats injected daily with refill liquid of electronic cigarette

containing nicotine concentration of 0.75 mg/kg for 5 weeks.

Electronic cigarette and vitamins (EC plus V) group: 8 rats injected daily 0.75 mg/kg nicotine of electronic cigarette liquid for 1 week. On day 8, they were supplied with Vit C and E combination by orogastric gavage, 1 hr after electronic cigarette liquid injection (for 4 weeks).

Nicotine (N) group: 8 rats were injected with 0.75 mg/kg nicotine daily for 5 weeks.

Nicotine and vitamins (N plus V) group: 8 rats injected daily 0.75 mg/kg nicotine for 1 week. On day 8, they were supplied with Vit C 300 mg/kg and Vit E 60 mg/kg by orogastric gavage, 1 hr after nicotine injection (for 4 weeks). (23–25)

At the start of this study, animals were weighed in order to calculate the dosage of nicotine, EC, vitamins C and E.

Methods:

Nicotine and electronic cigarette refill liquid administration:

Nicotine was purchased from (Sigma-Aldrich, Missouri, United States) and electronic cigarette liquid from commercial shops. Tobacco-flavored electronic cigarette refill liquids were used, with 12 mg/ml of nicotine. EC refill liquid contained propylene glycol, vegetal glycerin, flavorings and nicotine. Nicotinic administration continued 5 weeks with a concentration of 0.75 mg/kg/day dissolved in saline. (26,27) Both nicotine and EC refill liquids were freshly prepared each day and administered intraperitoneally once daily(28–31), always at the same time of the day for all animals.

The chosen nicotine dose corresponds to the daily nicotinic consumption of 10-20 cigarette rods. (27,30) Intraperitoneal (i.p.) mean of administration was used since it is easy, reliable and to exclude heat effects produced by smoking.(28,32)

Vitamins C and E administration:

The animals of EC plus V and Nicotine plus V groups were injected 0.75 mg/kg nicotine for 1 week to achieve nicotine dependence. After that, they were supplied with the combination of Vit C 300 mg/kg (23) (Chemical industries development, Giza, Egypt) and Vit E 60 mg/kg (24,25) (Pharco pharmaceuticals, Alexandria, Egypt) by orogastric gauge, 1 hr after each daily nicotine injection (for 4 weeks).

Vitamin C tablets were ground and mixed in distilled water. Vitamin E capsules inner components were mixed with extra virgin olive oil to prepare a suspension containing 60 mg of vitamin E. (24,25)

Tissue harvest:

All animals were sacrificed 1 day after the last injection by inhalation of an over- dose of diethyl ether. Tongues were dissected out, and divided horizontally into ventral and dorsal segments. The

ventral segments were used for light microscopic procedures.

Histological procedures:

Specimens for light microscopic examination were fixated by 10% formalin for 48 hours. Dehydrated process was done in ascending ethanol concentrations. Finally, specimens were infiltrated then embedded into paraffin wax. Sagittal sections of 5 μm thickness were obtained and stained with hematoxylin and eosin (H&E) for histological evaluation along with histomorphometric analysis.

Histomorphometric analysis:

The total surface area of oral epithelium in ventral surface of the tongue was quantified.

The values were measured from each animal from 2 different fields at 2 standardized depths. The measurements were estimated in photos captured using x100 magnification with the aid of image analysis system (Image J 1.53 version).(22)

Statistical analysis:

Kolmogorov-Smirnov test of normality showed no significance in variables distribution, thus parametric tests were used.(33) When F ratio of ANOVA was significant Levene test of homogeneity of variances was done, and if significant Brown-Forsythe Robust test was adopted. Games-Howell test was used in Post-hoc multiple comparisons. An alpha level was 5% with a significance level of 95%. Statistical significance was tested at p value ($<.05$).

RESULTS:

Histological observations of H&E-stained sections:
Control group exhibited normal thickness of the epithelium with intact basal layer and normal cellular organization. Normal structure of lamina propria was observed. Muscle fibers ran in different directions (fig.1). EC group revealed a thin layer of epithelium covered by keratin. The lamina propria was filled with engorged blood vessels (fig.2). The blood vessels present in lamina propria were dilated and showed thickened walls filled with eosinophilic amorphous material. (fig.3). EC plus V group showed regaining of keratin and underlying epithelial thickness. Moreover, preservation of normal organization of lamina propria was detected (fig.4). N group exhibited thinning of epithelium and keratin with complete absence of keratin covering layer in some regions. Lamina propria contained dilated engorged blood vessels. Areas of edema surrounding the blood vessels were seen (fig.5). N plus V group showed regaining of keratin and epithelium thickness. Lamina propria appeared of normal structure and devoid of dilated blood vessels (fig.6)

Histomorphometric analysis:

The total surface area (SA) of oral epithelium decreased significantly in EC (Mean \pm S.D.: 0.241 ± 0.055) and N (Mean \pm S.D.: 0.221 ± 0.053) groups. There was no significant difference

between control (Mean \pm S.D.: and both EC plus V (Mean \pm S.D.: 0.412 ± 0.081) and N plus V (Mean \pm S.D.: 0.411 ± 0.080) groups. (Table 1)

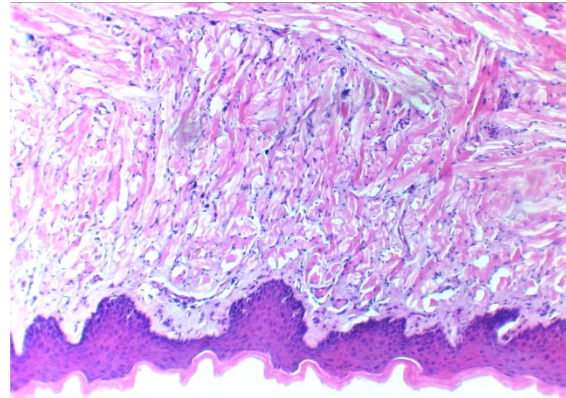


Figure 1: LM, (H&E stain) control, showing normal thickness of the epithelium with intact basal layer. Normal structure of lamina propria. (x100)

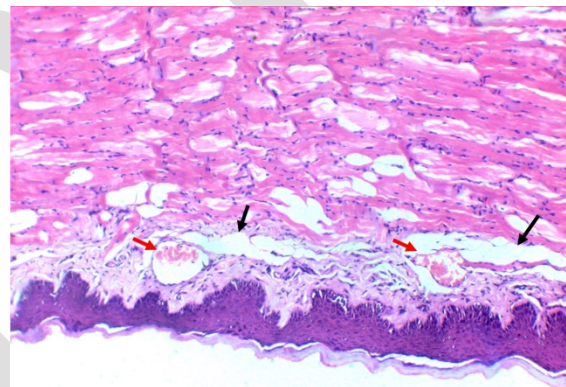


Figure 2: LM, (H&E stain), EC group, showing lamina propria is filled with engorged blood vessels (red arrow). Edema separating muscle fibers (black arrow) (x100)

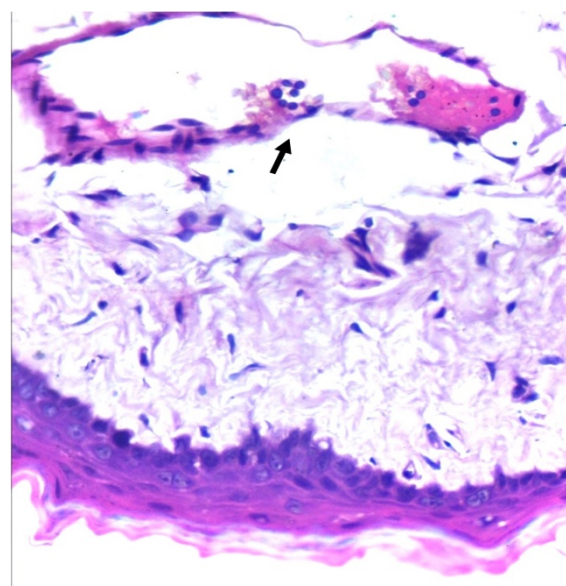


Figure 3: LM, (H&E stain), EC group, showing thin layer of epithelium covered by keratin. Note dilated blood vessel of thickened walls filled with eosinophilic amorphous material (black arrow). (x400)

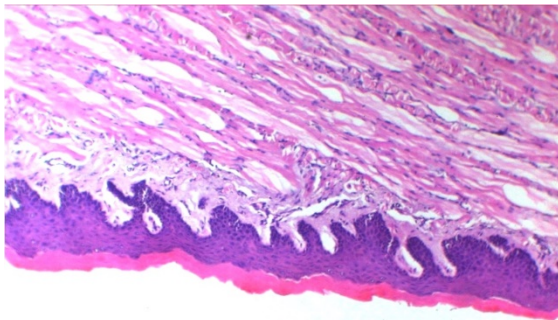


Figure 4: LM, (H&E stain), EC plus V group, revealing regaining of keratin and underlying epithelial thickness. (x100)

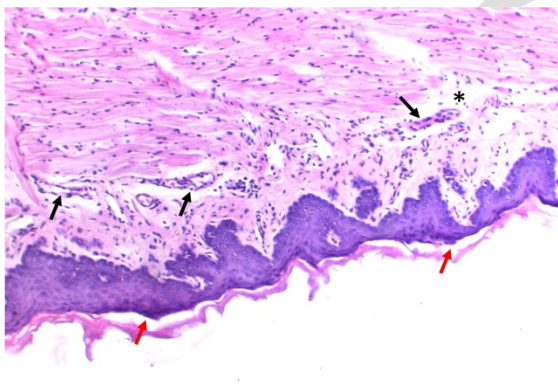


Figure 5: LM, (H&E stain), N group, showing thinning of epithelium and keratin with complete absence of keratin covering layer in some regions (red arrow). Lamina propria exhibited dilated engorged blood vessels (black arrow). Areas of edema surrounding the blood vessels were seen (asterisk). (x100)

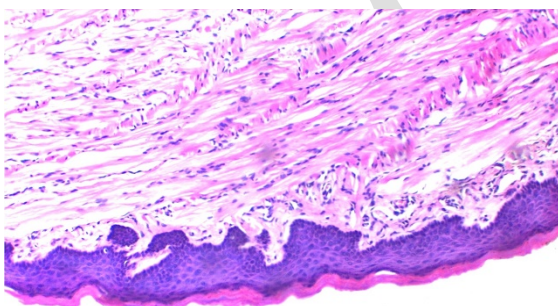


Figure 6: LM, (H&E stain), N plus V group, showing regaining of keratin and epithelium thickness. (x100)

Table 1: Average Total Surface Area Ventral Surface (mm²)

Average Total Surface Area Ventral Surface (mm ²)	Groups				
	Control	EC	EC plus V	N	N plus V
n	8	8	8	8	8
Min-Max	0.322-0.536	0.156-0.312	0.310-0.555	0.168-0.322	0.310-0.554
Mean	0.394	0.241	0.412±0	0.221	0.411
± S.D.	±0.06	±0.05	.081 ^{a,c,e}	±0.05	±0.08
S.E. of the mean	9 ^{a,c,e} 0.024	5 ^{b,d} 0.19	0.34436	3 ^{b,d} 0.019	0 ^{a,c,e} 0.028
95% CI for mean	0.336	0.194	-	0.176	0.344
Media	3-0.452	68-0.286	0.47902	51-0.264	25-0.478
[IQR]	07-0.452	57-0.286	[0.343-0.425]	86-0.264	62-0.478
	0.382	0.248	.0456]	0.206	0.415
	[0.341	-	-	[0.182	[0.343
	-	0.283]	-	-	-
	0.425]			0.251]	0.456]

Test of significance $F_{(df=4)} = 15.795$
 $p < .0001^*$
p

n : Number of animals
 Min-Max: Minimum – Maximum S.D.: Standard Deviation
 S.E. = Standard Error
 CI: Confidence interval. df: degree of freedom
p: probability of error (chance) (statistically significant if $<.05$)
 *: Statistically significant
 IQR: first to third quartile (25th to 75th percentile)
 Different letters indicate pairwise statistical significance using Games-Howell method

DISCUSSION:

The past 50 years witnessed the emergence of numerous studies and evidence documenting the hazardous health consequences of both active cigarette smoking and passive smoking by exposure to secondhand smoke. From 2016 to 2018, a significant increase was found in the prevalence of ECs smoking in the United States of America. This increase was mainly among middle-aged adults, as well as former smokers. (34)

A significant decrease in epithelial total surface area (epithelium and overlying keratin) was detected in rats injected with nicotine and electronic cigarettes. These results were consistent with Shahbaz et al. (35) who found a significant

decrease in nicotine smoking group when compared to control.

Another study published in 2018 showed decreased epithelial thickness, thinning of the overlying keratin layer, and irregular basement membrane after exposure to cigarette smoke. Moreover, the underlying connective tissue showed decrease in cellularity and exhibited numerous capillaries and blood vessels. (36) These findings were in accordance with the present study.

In contrast, the results discussed by Shammari et al. (37) along with Hamam et al. (38) were not in accordance with the current findings concerning the epithelial thickness. The previous studies detected thickening of the epithelium in smokers whereas, the present study observed obvious thinning in the epithelial coverage.

The present study exhibited multiple dilated engorged blood vessels in the lamina propria of electronic cigarettes and nicotine groups. In 2015, an experimental paper was conducted to study passive smoking effect on tongue blood vessels. The study showed abnormally dilated congested blood vessels, damage and loss of subintimal collagen layer.(39)In this regard, Zaheer et al. studied histological changes in rat buccal mucosa after nicotine injection. The results showed significant congestion and increase in vessel wall thickness: (40) This indicates the damaging effects on both epithelium and connective tissue.

Ekenedilichukwu et al. (12) and Karademircit et al. (41)found a significant decline in serum levels of both vitamin C and E when comparing smoking subjects to nonsmokers. Indeed, the results of Torshabi et al. showed that vitamins C and E caused a significant reduction in cytotoxicity of nicotine increased the viability of human gingival fibroblast cell line.(15) Vitamin C and E combination was chosen in the present study due to depletion of their levels and elevated turnover rate in smokers. Promising results were obtained, regarding supplementation of vitamins C and E antioxidants in reversing deleterious smoking effects and maintain the normal oral mucosal integrity and normal structure.

CONCLUSIONS:

Nicotine and electronic cigarettes have deleterious effects on the cellular organization of epithelium of ventral tongue surface. Supplementation of vitamins C and E together showed promising results in restoring the normal cellular architecture.

CONFLICT OF INTEREST:

No potential conflict of interest is present.

FUNDING:

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REFERENCE:

1. Kumar GS. Orban's Oral Histology and Embryology. 13th ed. Elsevier; 2011.
2. Carstens E, Carstens MI. Sensory Effects of Nicotine and Tobacco. *Nicotine & Tobacco Research*. 2021.
3. Patten T, Dreier A, Herman RJ, Kimball BA, De Biasi M. Exposure to fruit-flavoring during adolescence increases nicotine consumption and promotes dose escalation. *Neuropharmacology*. 2021;195:108672.
4. Schoenborn C, Gindi R. Electronic Cigarette Use Among Adults: United States, 2014 Key findings Data from the National Health Interview Survey. 2014.
5. Li X, Holahan CK, Holahan CJ. Sociodemographic and psychological characteristics of very light smoking among women in emerging adulthood, national survey of drug use and health, 2011. *Prev Chronic Dis*. 2015;12.
6. Pulvers K, Scheuermann TS, Romero DR, Basora B, Luo X, Ahluwalia JS. Classifying a smoker scale in adult daily and nondaily smokers. *Nicotine Tob Res*. 2013/12/01. 2014;16:591–9.
7. Prasetyaningtyas N, Jatiatmaja NA, Radithia D, Hendarti HT, Parmadiati AE, Hadi P, et al. The Response of the Tongue Epithelial on Cigarette Smoke Exposure as a Risk Factor for Oral Cancer Development. *European Journal of Dentistry*. 2021;15(2):320–4.
8. Wi MR, Michalska B, Drabik K, Prill M, Patalas-krawczyk P, Mathis C, et al. Mitochondria as a possible target for nicotine action. 260 *J Bioenerg Biomembr*. 2019;51:259–76.
9. Zhang W, Lin H, Zou M, Yuan Q, Huang Z, Pan X, et al. Nicotine in Inflammatory Diseases: Anti-Inflammatory and Pro-Inflammatory Effects. *Frontiers in Immunology*. 2022;13:1–21.
10. Welz C, Canis M, Schwenk-Zieger S, Becker S, Stucke V, Ihler F, et al. Cytotoxic and genotoxic effects of electronic cigarette liquids on human mucosal tissue cultures of the oropharynx. *Journal of Environmental Pathology, Toxicology and Oncology*. 2016;35:343–54.
11. Rouabhia M, Piché M, Corriveau MN, Chakir J. Effect of e-cigarettes on nasal epithelial cell growth, Ki67 expression, and pro-inflammatory cytokine secretion. *American Journal of Otolaryngology - Head and Neck Medicine and Surgery*. 2020;41:102686.
12. Ekenedilichukwu OJ, Chukwuemeka Solomon I, Chukwuemeka OE, Rosemary Adamma A, Onyema Athanatius O, Nwabunwanne OV, et al. The Effects Of Cigarette Smoking On The Serum Levels Of Some Antioxidants (Vitamin C And E) Amongst Male Smokers In Levels In College Of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. *International Journal of Scientific Research and Management*. 2018;6.
13. Raatz SK, Jahns L, Johnson LAK, Scheett A, Carriquiry A, Lemieux A, et al. Smokers report lower intake of key nutrients than nonsmokers, yet both fall short of meeting recommended intakes. *Nutrition Research*. 2017;45:30–7.
14. Prasad S, Kaisar MA, Cucullo L. Unhealthy smokers: Scopes for prophylactic intervention and

- clinical treatment. *BMC Neuroscience*. 2017;18:1–12.
15. Torshabi M, Rezaei Esfahrood Z, Jamshidi M, Mansuri Torshizi A, Sotoudeh S. Efficacy of vitamins E and C for reversing the cytotoxic effects of nicotine and cotinine. *European Journal of Oral Sciences*. 2017;125:426–37.
 16. Pozzer D, Invernizzi RW, Blaauw B, Cantoni O, Zito E. Ascorbic Acid Route to the Endoplasmic Reticulum: Function and Role in Disease. Vol. 34, *Antioxidants and Redox Signaling*. 2021. 845–855 p.
 17. Bogdan M, Meca AD, Boldeanu MV, Gheorghe DN, Turcu-Stiolică A, Subtirelu MS, et al. Possible involvement of vitamin C in periodontal disease-diabetes mellitus association. *Nutrients*. 2020;12:1–17.
 18. Anghel L, Baroiu L, Beznea A, Topor G, Grigore CA. The therapeutic relevance of Vitamin E. *Revista de Chimie*. 2019;70:3711–3.
 19. Arise RO, Olapade EO, Olufemi O, Yekeen AA. Taurine and vitamin E protect against pulmonary toxicity in rats exposed to cigarette smoke. *Sci Afr*. 2021;13:e00923.
 20. Asad Iqbal MD, Khan M, Kumar P, Kumar A, Ajai K. Role of vitamin e in prevention of oral cancer:-A review. *Journal of Clinical and Diagnostic Research*. 2014;8:ZE05–7.
 21. Radwan LRS, Grawish ME, Elmadawy SH, El-Hawary YM. Ultrasurface morphological changes in the rat tongue posterior one-third exposed to passive smoke. *EC Dental Science*. 2016;4:846–53.
 22. Abdelrahman M, Elsherbeny M. a Study To Detect the Safety of Electronic Cigarette on the Taste Buds of Albino Rats Tongue Papillae. *Egyptian Dental Journal*. 2019;65:1341–8.
 23. Mahmoud GS. Protective Effects of Vitamin C against Nicotine-Induced Oxidative Damage of Rat Liver and Kidney. *IOSR Journal of Environmental Science, Toxicology and Food Technology*. 2014;8:50–63.
 24. Bilbis LS, Muhammad SA, Saidu Y, Adamu Y. Effect of vitamins A, C, and e supplementation in the treatment of metabolic syndrome in albino rats. *Biochemistry Research International*. 2012;2012.
 25. Zakaria S, Mat-Husain SZ, Ying-Hwey K, Xin-Kai K, Mohd-Badawi A, Abd-Ghani NA, et al. Vitamin E improved bone strength and bone minerals in male rats given alcohol. *Iranian Journal of Basic Medical Sciences*. 2017;20:1360–7.
 26. Sodagar A, Donyavi Z, Arab S, Kharrazifard MJ. Effect of nicotine on orthodontic tooth movement in rats. *American Journal of Orthodontics and Dentofacial Orthopedics*. 2011;139:e261–5.
 27. Paunović MG, Ognjanović BI, Matić MM, Štajn AS, Saičić ZS. Protective effects of quercetin and Vitamin C against nicotine-induced toxicity in the blood of Wistar rats. *Arhiv za Higijenu Rada i Toksikologiju*. 2016;67:304–10.
 28. Milovanovic P, Stojanovic M, Antonijevic D, Cirovic A, Radenkovic M, Djuric M. “Dangerous duo”: Chronic nicotine exposure intensifies diabetes mellitus-related deterioration in bone microstructure - An experimental study in rats. *Life Sciences*. 2018;212:102–8.
 29. Ramalingam A, Santhanathas T, Ali SS, Zainalabidin S. Resveratrol supplementation protects against nicotine-induced kidney injury. *International Journal of Environmental Research and Public Health*. 2019;16.
 30. Bashiri H, Rezayof A, Sahebgharani M, Tavangar SM, Zarrindast MR. Modulatory effects of the basolateral amygdala α 2-adrenoceptors on nicotine-induced anxiogenic-like behaviours of rats in the elevated plus maze. *Neuropharmacology*. 2016;105:478–86.
 31. Hapidin H, Othman F, Soelaiman IN, Shuid AN, Luke DA, Mohamed N. Negative effects of nicotine on bone-resorbing cytokines and bone histomorphometric parameters in male rats. *Journal of Bone and Mineral Metabolism*. 2007;25:93–8.
 32. El.Golli N, Dkhili H, Dallagi Y, Rahali D, Lasram M, Bini-Dhouib I, et al. Comparison between electronic cigarette refill liquid and nicotine on metabolic parameters in rats. *Life Sciences*. 2016;146:131–8.
 33. Montgomery DC. *Design and analysis of experiments*. John wiley & sons; 2017.
 34. Szukalska M, Szyfter K, Florek E, Rodrigo JP, Rinaldo A, Mäkitie AA, et al. Electronic cigarettes and head and neck cancer risk—current state of art. *Cancers (Basel)*. 2020;12.
 35. Shahbaz M, Zaheer N, Sagheer A, Imran Arshad A, Zaheer U, Khursheed Alam M. Role of Green Tea Extract (*Camellia sinensis*) in Prevention of Nicotine-Induced Inflammatory and Epithelial Changes in Buccal Mucosa of Albino Rats. *International Medical Journal*. 2017;24:230–3.
 36. Ahmed S. Histological and Histomorphometric Evaluation of Anterior Part of Rats Tongue Exposed to Passive Cigarette Smoking. *Egyptian Journal of Oral and Maxillofacial Surgery*. 2018;9:174–8.
 37. Shammari AF Al, Ibrahim IK Al, Alaauldeen AI, Merza RF, Ahmed HG. Effects of Tobacco Smoking on the Dorsum of the Tongue and Buccal Epithelium. *Asian Pacific Journal of Cancer Prevention*. 2016;17:4713–8.
 38. Ghada Hamam and DEW. Effect of Cigarette Smoking on Human Gingival Mucosa-Histological and Morphometric Study. *Journal of Cytology & Histology*. 2018;09.
 39. Serwi Rasha Hamed, Elmeadawy Samah H, Radwan Lobna RS. Impact of passive smoke on tongue blood vessels, blood cell count and blood glutathione level (Experimental study). *EC Dental science*. 2015;423–34.
 40. Zaheer N, Shahbaz M, Athar Y, Arshad A, Zaheer U, Alam MK. Role of Green Tea Extract (*Camellia sinensis*) in Prevention of Nicotine induced Vascular Changes in Buccal Mucosa of Albino Rats. *Int Medical J*. 2017;24:120–5.
 41. Karademirci M, Kutlu R, Kilinc I. Relationship between smoking and total antioxidant status, total oxidant status, oxidative stress index, vit C, vit E. *Clinical Respiratory Journal*. 2018;12:2006–12.