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Effect of Maternal Nicotine Exposure on Circumvallate Papillae Development in Albino Rat

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Abstract

Background: Nicotine, the main ingredient in tobacco, readily crosses the placental barrier to cause deleterious effects on the health of the offspring. Objective: this study aimed to evaluate the potentially teratogenic effect of nicotine on the development of circumvallate papillae in pre and postnatal developmental periods Methodology: pregnant Albino rats were injected with nicotine hemisulfate at a dose of 1.6 mg/kg/day corresponding to the dose received during heavy smoking. Fetal tongue samples were collected at the 12th and 16th gestational days and postnatal samples were studied in newborns, at the age of 7 and 14 days of the off springs. Results: the main effects were distortion of the morphology of the papillae especially with advancing age in the postnatal stages, decrease in number of taste buds and distortion of its keratinization. Conclusion: it was concluded that the teratogenic effect of nicotine on circumvallate papillae seen as impaired development of its gustatory epithelium ensures its neurotoxic effect on peripheral nerves. The effect was long-lasting postnatally even when off springs were not subjected to nicotine.

Key words: Nicotine, Tongue papillae, Taste buds, scanning electron microscopy.

INTRODUCTION

Tobacco smoking is a global problem of civilization, with the number of tobacco smokers estimated at about 1.3 billion. Epidemiological studies show that one third of tobacco smokers die of cardiovascular diseases (Michcik *et al*., 2014). Statistically, the life expectancy of smokers is 15 years less than that of nonsmokers (Thun *et al*., 2012). Tobacco smoking not only increases the risk of systemic disease, but it also contributes to the risk of cancer genesis. Long-term exposure to carcinogens in the air is a high-risk factor for the development of lung cancer and oral cavity cancers (Agudo *et al*., 2012). Nicotine is an organic alkaloid compound found naturally throughout the tobacco plant. With each puff of a cigarette, a smoker pulls nicotine into his or her lungs where it is absorbed into the blood. Although the amount of nicotine inhaled with tobacco smoke is quite small, it is still sufficient to cause dependence. Thousands of researches have reported the deleterious effects of smoking on various tissues (Pappas 2011). Even passive smoking is hazardous because the side stream smoke contains high concentrations of ammonia, benzene, nicotine, carbon monoxide, and many carcinogens. It has been estimated that the total nicotine dose received by children whose parents smoke is equivalent to their actively smoking between 60 and 150 cigarettes per year (Holay *et al*., 2004). The passive exposure during childhood to parents smoking increased a child's risk of leukemia and lymphoma during adulthood (Hawamdeh *et al*., 2003).

Literature Review:

Since tobacco is a delivery system for the addictive agent nicotine, the dental profession has to perform oral examinations that focus on oral cancer detection and the other oral changes occurring with tobacco use (Taybos 2003). The effects of nicotine on epithelial cells of the respiratory tract are well documented, but the oral cavity has not been so well explored. The results of a few studies comparing the morphology of the oral epithelium of smokers with that of nonsmokers pointed to an increase in its thickness and also to higher levels of cytokeratin in smokers' cells (Pickett *et al*., 2010). It is well known that epithelia exposed to adverse chemical or physical effects defend themselves by increasing cytokeratin levels (Shaykhiev *et al*., 2011). Many studies have been concerned with the mechanism of action of nicotine in the etiology of oral mucosal disease (Gültekin *et al*., 2008). Tobacco smoke comes into direct contact with oral tissues and is responsible for delivering an array of chemical pollutants in to the buccal cavitysuchasnicotine, carbon monoxide, and hydrogencyanide. Smoking has avasoco nstrictive effect on thegingivalmicrocirculationresultinginreducedbloodflowingingivaltissues. It has been observed that significantly increased levels of TNF-a have been detect eding in givalcrevi cularfluid in both current and former smokers who have received treatment or no treatment for periodontal disease (Eratilla *et al*., 2016).

Numerous experimental and clinical studies have determined the adverse effects of nicotine on the growth factors (Mathur RS., *et al*; 2000). It has been found that nicotine impaired epidermal growth factor mediated cellular functions in the rat buccal mucosa. To clarify the effects of nicotine on the keratinization of oral mucosal and epidermal keratinocytes, it was reported that nicotine stimulated differentiation of both mucosal and epidermal keratinocytes, and this nicotine-induced abnormal differentiation might be associated with the development of oral white lesions (Kwon OS., *et al.*, 1999). It was reported that systemic administration of nicotine damaged the cellular integrity of the oral mucosa, impairing tissue function and predisposing the tissue to the action of different pathogenic agents (Caldeira C., *et al.*, 2006).

Prenatal exposure to nicotine leads to growth retardation, impaired development of the nervous system, particularly the forebrain, as well as the branchial arches possibly leading to microcephaly and cleft palate respectively (Muhammad A., *et al.*, 2012). It was reported that maternal smoking during pregnancy and postnatal exposure to environmental tobacco smoke were predisposition factors to acute respiratory infections (Jedrychowski and Flak1979). Studies on human and animal models have revealed that prenatal exposure to nicotine during a critical prenatal period of brain development resulted in significant reduction of postnatal body weight, delay in appearance of body hairs, and significant delay in eye opening sensory motor reflexes(Gibbs K, *et al*;2016). It was also stated that maternal smoking during the second trimester was associated with fetal growth restriction and the degree of risk of smoking during pregnancy may reach the

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predisposition of spontaneous abortion (Windham GC., *et al.*1999). Striking teratogenic effects of nicotine were observed in the skin development in neonatal rats. Increased mitotic activity was noticed in the basal cells and hypertrophic epithelial cells were prominent in the epidermis. This study supported the concept of the teratogenic effects of nicotine on the epithelium (Inaloz HS., *et al.*, 2000).

Regarding the teratogenic effects of nicotine on oral tissues especially on epithelial tissues, it was found that foetuses of pregnant mice, exposed to intraperitoneal injection of nicotine sulfate, 9.6% of foetuses of nicotine injected mothers presented clefts of the palate (Saito T., *et al*.2005). It has been found that the developing molars of the foetuses of nicotine injected mothers were retarded, less differentiated, and reduced in breadth in comparison with controls. It is suggested that nicotine, or its metabolic by-products, interfere with normal interaction between the epithelial and mesenchymal components of the developing tooth (Chowdhury and Bromage 2000) The effect of nicotine on tongue development of mouse have shown that 6% of the nicotine-treated foetuses at the eighteenth gestational day had palatal clefts and their tongue development was much retarded compared to the controls. The tongues of the clefted foetuses were misshaped, reduced in size, had no filiform or fungiform papillae, and their myotubes were just in the process of formation. The circumvallate papilla of these foetuses was present but neither taste buds nor glands of von Ebner had as yet developed (Tomassini S., *et al*., 2007).

Objective:

This study aimed to evaluate the effect of nicotine on the prenatal and postnatal development of the circumvallate papillae of Albino rats at different stages of development

MATERIAL AND METHODS

This study was conducted on 66 tongues of Albino rats of both sexes, which were collected at day 12 and 16 of prenatal development (E) and at day 7 and 14 of postnatal life (P). Thirty-two timed pregnant albino rats were maintained in controlled environment. The date of vaginal plug formation was assumed to be day 0 of gestation (Ochiogu, *et al.*, 2006). Rats were assigned to one of two groups: the experimental group (n=20) received daily intraperitoneal injection of 0.1% nicotine hemi sulfate " N.H.S. "1.67 mg/kg of body weight (Gartner *et al.* 1997) from the 6th gestational day till giving birth or date of scarification. Their fetuses and off springs were considered the experimental group exposed to maternal nicotine (N). The control groups (n=12) were injected with an equivalent volume of sterile saline. The experimental group was further divided into four subgroups of five rats each. Fetuses were removed from pregnant rats – three from each rat-at two embryonic stages with the following labels: Group NE12: fifteen fetuses were removed at 12th gestational day. Group NE16: fifteen fetuses were removed at 16th gestational day. Tongues were removed from born rats at two postnatal ages: Group NP7: fifteen off springs sacrificed at age of 7 days. Group NP14: fifteen off springs sacrificed at age of 14 days.

For the control groups, which contained three rats each, tongue samples were collected from fetuses and off springs at matched embryonic stages and postnatal ages as the experimental group sand were labelled as follows: Group E12: nine fetuses were removed at 12th gestational day. Group E16: nine fetuses were removed at 16th gestational day. Group P7: nine off springs which were sacrificed at age of 7 days. Group P14: nine off springs which were sacrificed at age of 14 days. Fetuses were removed on the specified dates from the pregnant female rats just after merciful sacrifice by cervical dislocation. Tongues were dissected from rat fetuses and off springs immediately after scarification. The tongues were processed for light and SEM examination.

The effects of N.H.S. on the development of circumvallate papillae with respect to the number of taste buds was assessed by counting the number of taste buds using light microscopic slides for groupsNP14 and P14.

Data was tabulated and analyzed using Student's t test, which was performed using SPSS. software.

Results:

Embryonic stage:

At 12th gestational day: a) Control group (E12): Before that date of the embryonic period, no circumvallate papilla were observed to develop. The first detectable evidence of its development was at the 12th gestational day when it appeared as a circular elevation on the tongue surface with smooth surfaced central papillary part and the surrounding furrow just started to develop but no distinguishable flanking papillary structure (fig.1). b) Experimental group (NE12): The circumvallate papilla appeared oval with wrinkled surface, no distinguishable flanking papillary structure and the surrounding furrow started to develop (fig.2).A16th gestational day) Control groups (E16): The rounded circumvallate papilla increased in size and the elevated flanking papillary structure was formed surrounding the central papillary part from behind with a horseshoe like profile (fig.3). b) Experimental groups (NE16): There was an increase in size of the oval circumvallate papilla more than that seen in group NE12 and also showed a wrinkled surface and no flanking papillary structure was observed all around (fig.4).

Postnatal stage:

At 7 days of age: a. Control groups (P7): In group P7, no observed difference from group P0 except for the increase in size of the papillae (fig. 5).b. Experimental group (NP7): In group NP7, the papillary surface was more regular than group NP0 but there was apparent tearing of keratin in certain areas (fig.6). At 14 days of age: a) Control groups (P14): In group P14, no difference from group P7 was observed except for appearance of surface microridges (fig.7). b) Experimental group (NP14): In subgroup NP14, the circumvallate papilla appeared with distorted morphology and with winkled surface (fig.8).

Statistical results:

There was a highly significant difference in the mean of number of taste buds between control group (P14) and experimental group (NP14) (P<0.001). (table 1).

Table 1: Comparison between group P14 and NP14 regarding number of taste buds in circumvallate papilla.

Group	Mean $\pm$ SD	t	P
Control (P14)	22.00 $\pm$ 4.00	6.32	<0.001
Experimental (NP14)	9.80 $\pm$ 4.36		

SD= standard deviation, t= Student's t test value.
P>0.05=non significant, P$\leq$0.05=significant and P$\leq$0.001=highly significant.

Discussion:

Tobacco products are commonly used as non-medicinal drugs by pregnant women and are known to cause various effects on the fetus and the newborn. One of the most important ingredients of tobacco is nicotine which is a well-known toxic alkaloid substance with several teratogenic effects on the developing systems; it causes decreased fetal weights, hydrocephaly, delayed ossification of the bones of the skull, defective development of skeletal muscles, as well as teratogenic effects on oral mucosa (Gartner *et al*. 1997).Very few studies have been concerned with the deleterious effects of nicotine on the tongue development especially the development of the lingual papillae which was the concern in this work.

In the present study, the female rats of the experimental groups were injected with 0.1% N.H.S. 1 mg/kg of body weight once daily from the 6th gestational day till birth of off springs, this dose is equivalent to almost twenty-five cigarettes per day (Gartner *et al*.1997). SEM was used to demonstrate the surface characteristics and the three-dimensional picture of the morphogenesis of the papillae in various stages of development starting from embryogenesis to early postnatal stages. The date of initiation of circumvallate papilla development was detected as early as the 12th gestational day illustrated by epithelial thickening on the dorsal tongue surface. This was in accordance with (Suwanna *et al*., 2002) who added that the presence of the epithelial thickening in the location of the future vallate papilla at that date was followed by arrival of the innervation in the 13th gestational day.

In the present study, the early development of the lining epithelium of the dorsal surface of the lingual body was affected in experimental animals as early as in the 12th intrauterine day (group NE12) where light microscopic examination revealed that the surface epithelial cells were separated in several areas which

might be an indication of ulceration; many surface detachments were noticed on the scanning electron micrographs of tongue surfaces of group NE12 which ensured the presence of mucosal ulcerations and surface cell death. These findings are in accordance with earlier studies investigating the effect of systemic administration of nicotine on rat cheek mucosa using light and transmission electron microscopy which showed damage of the cellular integrity of the oral mucosa, impairing tissue function and predisposing the tissue to the action of different pathogenic agents (Caldeira EJ., *et al*., 2007).

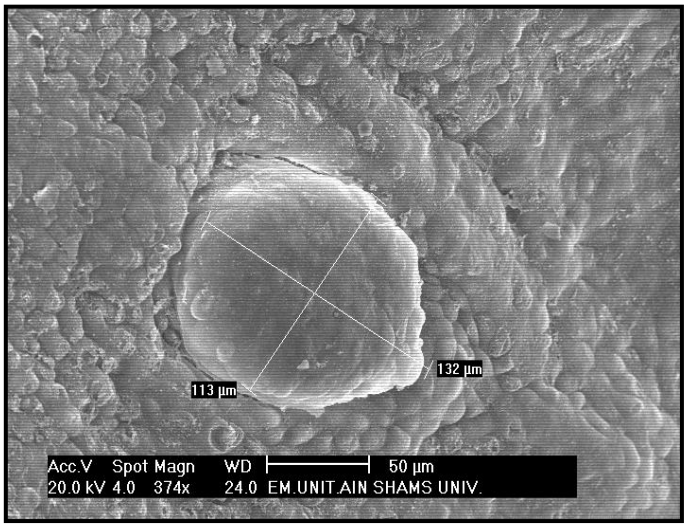

Fig. 1: A scanning electron micrograph of group E12 showing circular circumvallate papilla with smooth surfaced central papillary part but no distinguishable flanking papillary structure (x 374).

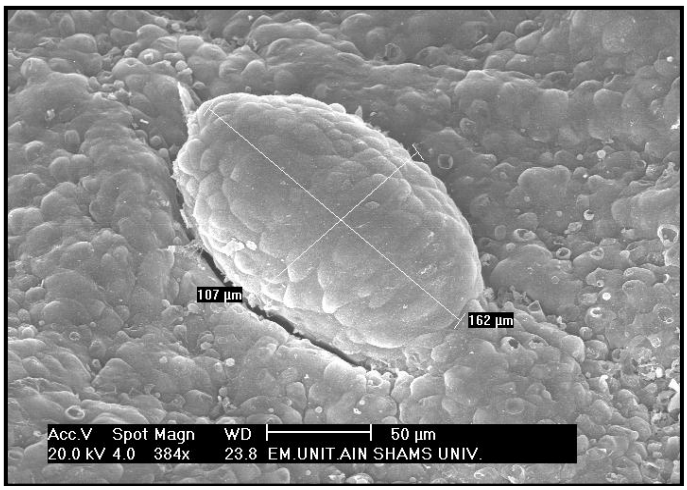

Fig. 2: A scanning electron micrograph of group NE12 showing the developing oval circumvallate papilla with wrinkled surface and no observed flanking papillary structure (x 384).

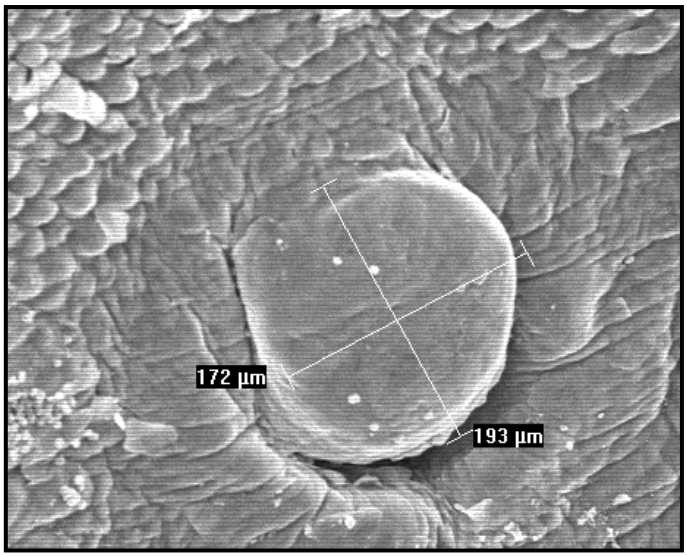

Fig. 3: A scanning electron micrograph of group E16 showing the developing spherical circumvallate papilla with flanking papillary part from behind (x 400).

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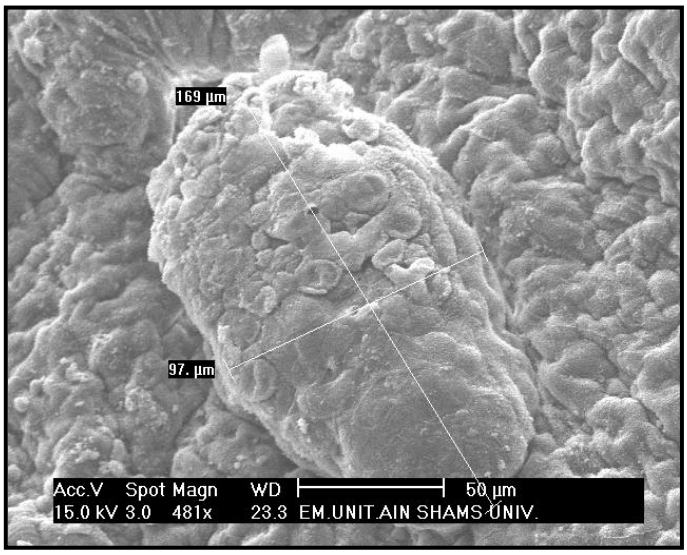

Fig. 4: A scanning electron micrograph of group NE16 showing oval circumvallate papilla with wrinkled surface and no observed flanking papillary structure (x 481).

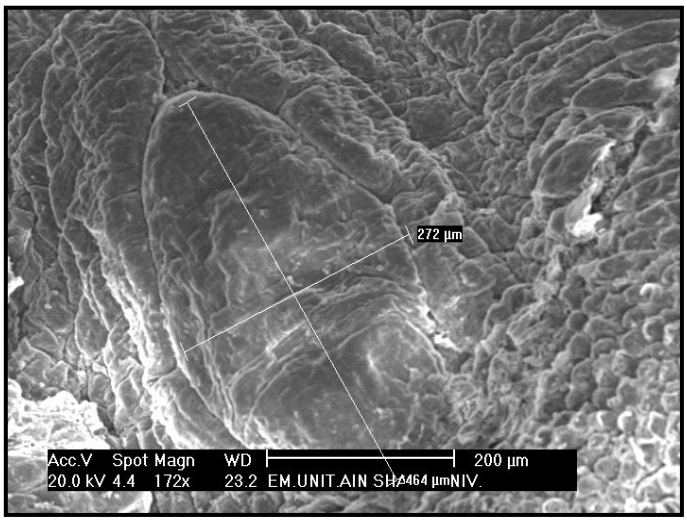

Fig. 5: A scanning electron micrograph of group P7 showing the circumvallate papilla which tends to be oval (x 172).

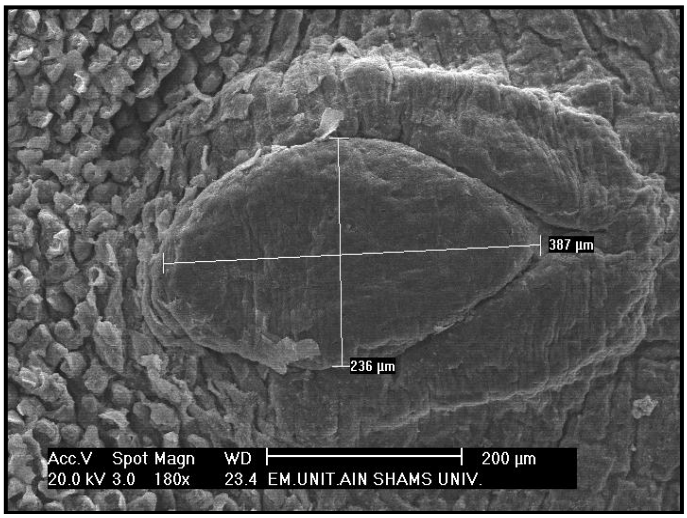

Fig. 6: A scanning electron micrograph of group NP7 showing the circumvallate papilla. Some desquamated scales were found (arrows) (x 180)

Many studies related the mechanism of the epithelial damage and mucosal ulcers caused by nicotine or tobacco smoking to its reduction of the salivary epidermal growth factor level and impairment of the function of buccal epidermal growth factor receptor, which might be associated with the pathology of smoking-related oral disease. (Eastwood GL., *et al*., 1997) Hence the adverse effect of nicotine on the quality of mucosal protective factors was a concluded to be an important contributor to the pathogenesis of peptic ulcer disease. This hypothesis was confirmed by (Maity., *et al*., 2003)who reported that "the gastric mucosal

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integrity is maintained by interplay of some aggressive and defensive factors controlling apoptotic cell death and cell proliferation and smoking potentiates ulcer by disturbing this balance".In the prenatal stages of circumvallate papilla development, the delayed initiation of trench development till 16th day intrauterine might be attributed to the effects of nicotine on growth factors and increased apoptosis and cell necrosis In the postnatal stages, some of the circumvallate papillae were distorted and covered by poorly developed nonkeratinized epithelium but many were covered by keratinized stratified squamous epithelia which were thin and with thin or absent keratin in many areas. These defects in the epithelium and keratin could be attributed to effects of nicotine on growth factors, apoptosis and mucosal protective factors (Li l.f., *et al*., 2014).The predisposition of mucosal ulceration by nicotine was reported in other mucosal regions such as gastric and duodenal mucosa, where cigarette smoking was reported to decrease gastric acid secretion and gastric mucosal blood flow causing peptic ulcers (Parasher and Eastwood 2000).

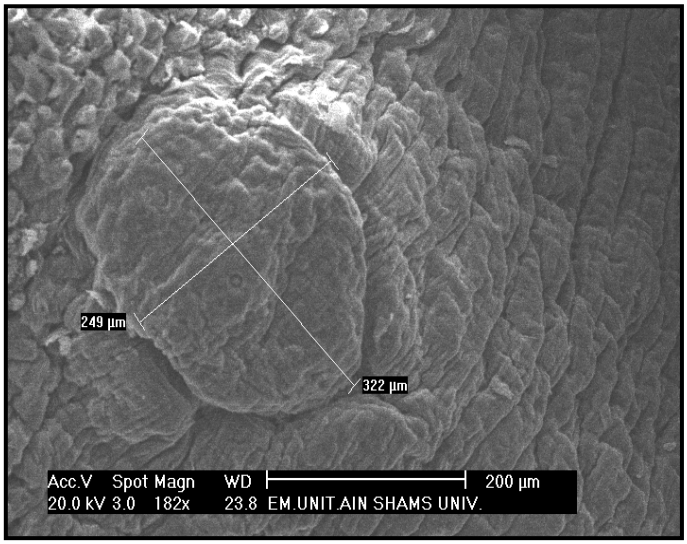

Fig. 7: A scanning electron micrograph of group P14 showing the circumvallate papilla (x 182).

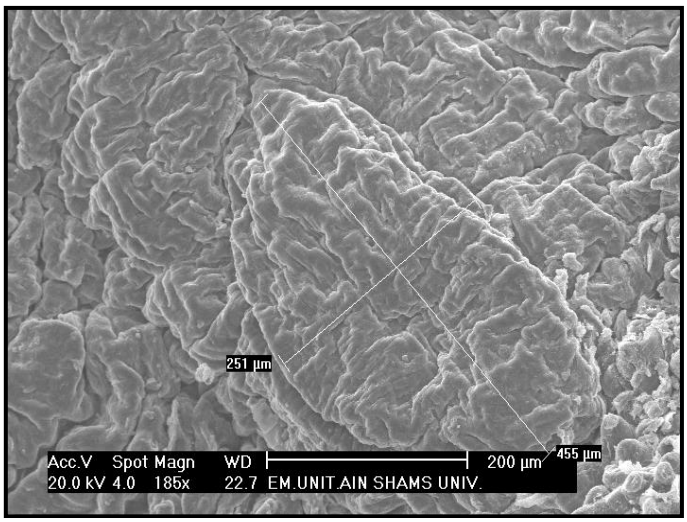

Fig. 8: A scanning electron micrograph of group NP14 showing the circumvallate papilla. The papilla is oval and distorted (x 185).

Another important effect of nicotine on circumvallate papilla noticed in this study was the decrease in number of taste buds in all stages of development of the circumvallate papilla, this decrease was statistically highly significant in group NP14. This finding is in agreement with (Gartner *et al*.1997) who studied the effect of nicotine on tongue development in mice, it was stated that the circumvallate papillae were present but without any taste buds. The absence of taste buds from the developing circumvallate papillae in that study could be attributed to the early date (18th gestational day) when the author examined the fetuses' tongues. Another cause might be the higher nicotine dose (1.76 mg/kg) administered to the experimental group in that study. Moreover, this result coincides with that reported by (Reiter *et al*. 2006) who stated that nicotine is considered a toxic substance that might cause taste dysfunction due to its effects on the gustatory system starting from the taste bud to the central neural pathways. The adverse effect of nicotine on the size, morphology and taste buds number of circumvallate papilla could be attributed to the previously reported adverse effects of nicotine on the growth factors This explanation is in agreement with (Mistretta *et al*., 1999) who reported that tongues of brain derived nerve growth factor null mutant mice, the circumvallate papilla was reduced in diameter and length and papilla morphology was disrupted. Taste bud number in the circumvallate was reduced by about 70% in mutant tongues, and the remaining taste buds were smaller than those on intact mice tongues.

The development of taste bud under influence of taste sensory innervation has been reported to be an important step in the development of taste bud containing papillae namely fungiform, circumvallate and foliate as a study examining the tongues of mice fetuses of which the amniotic cavities were injected by beta-bungarotoxin (neurotoxin) showed that no taste buds were associated with circumvallate papillae in the injected mice (Morris-Wiman J., *et al*., 1999). The effect of nicotine on the development of taste buds might be attributed to the deleterious effect on the development of nervous tissues and innervation of the gustatory epithelia which are necessary for proper development of taste buds (Northcutt RG. 2005). In a study conducted by (Vaglenova *et al*., 2004), it was found that prenatal nicotine exposure produced significant long-term developmental and behavioral teratogenic effects on the neural tissues, so the authors considered that nicotine is a neuroteratogen, specifically targeting brain development. The results seen in the off springs in the present study could be considered as continuation of the effects of nicotine on the development of filiform papillae, but this indicated that nicotine crossed the placenta and is still present in the blood of the off springs causing long term effects since we didn't inject the off springs with nicotine in our work. Nicotine was reported as one of the drugs that cross the placenta, most frequently used by pregnant women and has deleterious effects on the fetus and neonate and has long-term medical problems (Wickström R. 2007). Moreover, the improper development of the gustatory lingual epithelium in the experimental groups might be due to the effect of nicotine on the growth factors as

discussed before. This conclusion was supported by studies that showed that development of anterior gustatory epithelia in the palate and tongue requires epidermal growth factors; and reported that epidermal growth factor receptor deficiency selectively impaired the development of anterior gustatory epithelia in the mouth. (Robin F.2007).

Conclusion:

It was concluded that maternal nicotine intaketo pregnant female Albino rats had teratogenic effects on development of the lingual circumvallate papillae which extended even in the postnatal development period. These results indicate that nicotine might have the same harmful effects on humans which should urge the pregnant females to stop smoking. More research work is required to examine the effects of nicotine on the ultrastructure of these papillae using transmission electron microscopy. Also, further studies are needed to reveal the mechanisms causing these effects of nicotine on lingual papillae and to demonstrate these effects throughout later stages of the rat life in order to evaluate the long-term effects of nicotine on the papillae and whether these effects are reversible or not.

REFERENCES

Agudo, A., C. Bonet, N. Travier, 2012. Impact of cigarette smokingon cancer risk in the European prospective investigation intocancer and nutrition study. J Clin Oncol, 30: 4550-4557.

Caldeira, C., C. Carvalho, J. Padovani, P. Camilli, V. Garcia, Cagnon, 2006. Morphological alterations in the epithelium of the oral mucosa of rats (Rattus norvegicus) submitted to long-term systemic nicotine treatment. Archives of Oral Biology, 52(1): 83-89.

Caldeira, E.J., C.A. Carvalho, C.R. Padovani, J.A. Camilli, P.J. Garcia and V.H. Cagnon, 2007. Morphological alterations in the epithelium of the oral mucosa of rats (Rattus norvegicus) submitted to long-term systemic nicotine treatment. Arch Oral Biol., 52(1): 83-9.

Chowdhury, I.G., T.G. Bromage, 2000. Effects of fetal exposure to nicotine on dental development of the laboratory rat Anat Rec., 2581(4): 397-405.

Eastwood, G.L., 1997. Is smoking still important in the pathogenesis of peptic ulcer disease. J. Clin. Gastroenterol, 25(1): S1-7.

Gartner, L.P., A.Y. Saad, J.L. Hiatt, 1997. Effects of nicotine on tongue development in the CD-1 mouse. Eur. J. Morphol., 35(5): 337-43.

Gibbs, K., J.M. Collaco, S.A. McGrath-Morrow, 2016. Impact of Tobacco Smoke and Nicotine Exposure on Lung Development. Chest,149(2): 552-561.

Gültekin, S.E., B. Sengüven, B. Karaduman, 2008. The effect of smoking on epithelial proliferation in healthy and periodontally diseased marginal gingival epithelium. J. Periodontol., 79(8): 1444-50.

Hawamdeh, A., F.A. Kasasbeh, M.A. Ahmad, 2003. Effects of passive smoking on children's health: a review. East Mediterr Health J., 9(3): 441-7.

Holay, M.P., N.P. Paunikar, P.P. Joshi, V.S. Sahasrabhojney, S.R. Tankhiwale, 2004. Effect of passive smoking on endothelial function in: healthy adults. J Assoc Physicians India, 52: 114-7.

Inaloz, H.S., S.S. Inaloz, E. Deveci, A. Eralp, 2000. Teratogenic effects of nicotine on rat skin. Clin Exp Obstet Gynecol, 227(3-4): 241-3.

Jedrychowski, W., E. Flak, 1979. Maternal smoking during pregnancy and postnatal exposure to environmental tobacco smoke as predisposition factors to acute respiratory infections. Environ Health Perspect, 105(3): 302-6.

Kwon, O.S., J.H. Chung, K.H. Cho, D.H. Suh, K.C. Park, K.H. Kim, H.E. Eun, 1999. Nicotine-enhanced epithelial differentiation in reconstructed human oral mucosa in vitro. Skin Pharmacol Appl Skin Physiol., 12(4): 227-34.

Li, L.f., R.L.Y. Chan, L. Lu, J. Shen, L. Zhang, W.K.K. Wu, L. Wang, T. Hu, M.X. Li and C.H. Cho, 2014. Cigarette smoking and gastrointestinal diseases: The causal relationship and underlying molecular mechanisms (Review). International Journal of Molecular Medicine, 34: 372-380.

Maity, P., K. Biswas, S. Roy, R.K. Banerjee, U. Bandyopadhyay, 2003. Smoking and the pathogenesis of gastroduodenal ulcer--recent mechanistic update. Mol Cell Biochem., 253(1-2): 329-38.

Mathur, R.S., S.P. Mathur, R.C. Young, 2000. Up-regulation of epidermal growth factor-receptors (EGF-R) by nicotine in cervical cancer cell lines: this effect may be mediated by EGF. Am. J. Reprod Immunol., 44(2): 114-20.

Morris-Wiman, J. E. Basco and Y. Du, 1999. The effects of beta-bungarotoxin on the morphogenesis of taste papillae and taste buds in the mouse. Chem. Senses, 24(1): 7-17.

Muhammad, A., R. Mychasiuk, A. Nakahashi, S.R. Hossain, R. Gibb, B. Kolb, 2012. Prenatal nicotine exposure alters neuroanatomical organization of the developing brain. Synapse, 66(11): 950-4.

Mistretta, C.M., K.A. Goosens, I. Farinas and L.F. Reichardt, 1999. Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. J. Comp. Neurol., 409(1): 13-24.

Michcik, A., M. Cichorek, A. Daca, P. Chomik, S. Wojcik, A. Zawrocki, A. Wlodarkiewicz, 2014. Tobacco smoking alters the number of oral epithelial cells with apoptotic features. Folia Histochem Cytobiol, 52(1):60-68.

Northcutt, R.G., 2005. Taste buds: development and evolution. Brain Behav. Evol., 64(3):198-206.

Ochiogu, Izuchukwu Shedrack, Uchendu, Chukwuka Nwocha and Ihedioho, John Ikechukwu, 2006. A new and simple method of confirmatory detection of mating in albino rats (Rattus norvegicus). Animal Research International, 3(3): 527-530.

Pappas, RS., 2011. Toxic elements in tobacco and in cigarettesmoke: inflammation and sensitization Metallomics, 3: 1181-1198.

Pickett, G., J. Seagrave, S. Boggs, G. Polzin, P. Richter, Y. Tesfaigzi, 2010. Effects of 10 cigarette smoke condensates on primary human airway epithelial cells by comparative gene and cytokine expression studies. Toxicol Sci., 114: 79-89.

Parasher, G. and G.L. Eastwood, 2000. Smoking and peptic ulcer in the Helicobacter pylori era. Eur J Gastroenterol Hepatol.,12: 843-853.

Reiter, E.R., L.J. Dinardo and R.M. Costanzo, 2006. Toxic effects on gustatory function. Adv. Otorhinolaryngol, 63:265-77

Robin, F., 2007. Factors that regulate embryonic gustatory development Review BMC Neuroscience, 8(Suppl 3): S4 doi:10.1186/1471-2202-8-S3-S4.

Saito, T., X.M. Cui, T. Yamamoto, N. Shiomi, P. Bringas, Jr, C.F. Shuler, 2005. Effect of N'-nitrosonornicotine (NNN) on murine palatal fusion in vitro. Toxicology, 28: 207(3):475-85.

Shaykhiev, R., F. Otaki, P. Bonsu, 2011. Cigarette smoking reprograms apical junctional complex molecular architecture in the human airway epithelium in vivo. Cell Mol Life Sci., 68: 877-892.

Suwanna, J., C. Yang and Malcolm, L. Snead, 2002. Developmental patterning of the circumvallate papilla. Int. J. Dev. Biol., 46: 755-763.

Taybos, G., 2003. Oral changes associated with tobacco use. Am. J. Med. Sci., 326(4): 179-82.

Tomassini, S., V. Cuoghi, E. Catalani, G. Casini and A. Bigiani, 2007. Long-term effects of nicotine on rat fungiform taste buds. Neuroscience, 147(3): 803-10.

Thun, M., R. Peto, J. Boreham, A.D. Lopez. 2012. Stages of the cigaretteepidemic on entering its second century, Tob., 21: 96-101.

Vaglenova, J., S. Birru, NM. Pandiella, C.R. Breese, 2004. An assessment of the long-term developmental and behavioral teratogenicity of prenatal nicotine exposure. Behav Brain Res., 150(1-2): 159-70.

Windham, G.C., J. Von Behren, K. Waller, L. Fenster, 1999. Exposure to environmental and mainstream tobacco smoke and risk of spontaneous abortion. Am. J. Epidemiol., 149(3): 243-7.

Wickström, R., 2007. Effects of nicotine during pregnancy: human and experimental evidence. Curr. Neuropharmacol, 5(3): 213-22.