

## The Effects of Nicotine on the Incisive Teeth and Expression of Vimentin in Rats

Efectos de la Nicotina sobre los Dientes Incisivos y la Expresión de Vimentina en Ratas

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**SUMMARY:** Nicotine is an alkaloid toxic effects of oral cavity. In this study, 14 adult Sprague-Dawley rats weighing 230-260 mg ( $\pm 10$  mg) were used as experimental animal. The rats of experimental group (n=6) were nicotine systemically with nicotine sulphate, 2mg/kg subcutaneously, daily in period of 28 days. Pulp, alveolar bone tissue, periodontal membrane and gingival epithelial junction were investigated in these regions in incisive teeth longitudinal cross-section. Thinning of the collagen fibers in the pulp tissue, vascular congestion, and inflammatory cell infiltration were observed. Mesenchymal tissues that is stained positive for vimentin lay underneath the epithelium. A strong expression of vimentin can be observed in formed periodontal ligament.

**KEY WORDS:** Nicotine; Rat; Vimentin.

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### INTRODUCTION

Nicotine is an alkaloid from the oxygen-free alkaloid group, which is the major psychoactive component of cigarette smoke that is highly addictive if inhaled via binding to nicotinic cholinergic receptors in the brain and releasing neurotransmitters such as dopamine and by causing neuroadaptation (Henningfield & Goldberg, 1988). Many studies have investigated nicotine's cellular and molecular effects. Investigations of nicotine's effect on cultured cells suggest that it can influence bone resorption and apposition (Yuhara *et al.*, 1999). Nicotine causes an increase in the expression of the cyclo-oxygenase-2 (COX-2) gene and prostaglandin E2 (PGE2) release in human gingival fibroblasts in a time and dose-dependent manner (Nakao *et al.*, 2009). Paulson *et al.* (2009), demonstrated a direct relationship between nicotine dose and decrease of the fetal weight, number of resorptions and malformations, embryotoxicity and intrauterine growth retardation. It is now accepted that an important causing factor of intrauterine growth retardation in smoking mother's conceptuses is fetal hypoxia and/or ischemia due to reduction of the uteroplacental blood flow by both carbon monoxide and nicotine of the cigarette (Donnenfeld *et al.*, 1993). The present investigation revealed that cigarette smoke might

provoke alterations at cellular level, even in a passive absorption.

### MATERIAL AND METHOD

The study protocol was approved by the Animal Research Committee of Dicle University, Turkey. 14 adult Sprague-Dawley rats weighing 230-260 g ( $\pm 10$  g) were used as experimental animal. The animals were group-housed (7 per cage) under standard conditions in the Animal Health and Research Center of Dicle University. The animals were fed *ad libitum* with water and standard laboratory animal diet, under the care of trained wardens. The rats were divided into 2 groups as: The rats of experimental group (n=6) were nicotine systemically with nicotine sulphate (Sigma, Aldrich), 2mg/kg subcutaneously, daily in period of 28 days. The rats of group control (n=6) was used as control and did not receive NIC, but were maintained in similar environment and food.

**Tissue Preparation for Light Microscopy.** At the end of the study, animals were sacrificed decapitation. The maxillary

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regions were dissected under ketamine hydrochloride anesthesia. The samples were fixed with neutral buffered formalin solution and decalcified with 5% EDTA (Ethylene-diamine-tetraacetic acid). After preservation, nasal samples were directly dehydrated in a graded series of ethanol and embedded into paraffin wax. 4-6 mm sections were cut with microtome (Rotatory Microtome, Leica, RM 2265, Germany) and mounted on the coated slides. The sections were stained with H-E, and Trichrome-Masson in order to be observed under light microscope (Nikon Eclipse 80i).

**Immunohistochemistry stain.** Phosphate-buffered solution (PBS, pH 7.2) were washed 3x5 minutes. Citrate buffer, tissue sections were antigenically masked for the abolition of the environment and 2x5 minutes in a microwave oven, waited outside for 10 minutes. The slides were washed in PBS, and marked the boundaries of tissue on the hydrophobic pen. Three percent hydrogen peroxide were dropped on the tissue and allowed to stand for 15 minutes. Sections were washed in PBS at room temperature and humid 80 min were stained with antibodies to vimentin in the primary. Washed with PBS. Secondary antibodies and streptavidin-peroxidase complex, 30 minutes, stained. Diaminobenzidine as the chromogen (DAB) were stained for 3-5 minutes. One minute with Harris hematoxylin staining was the opposite.

## RESULTS

Histological analysis of nicotine-treated group and the control group were compared with the incisive teeth. Pulp, alveolar bone tissue, periodontal membrane and gingival epithelial junction were investigated in these regions, incisive teeth longitudinal cross-section, thinning of the collagen fibers in the pulp tissue, vascular congestion, and inflammatory cell infiltration were observed (Figure 1A). Periodontal membrane, expansion of blood vessels, red blood cells showed intense perivascular infiltration (Figure 1B). Fibroblast cells, collagen fibers, characterized by hyperplasia of the intermediate stroma expansion was seen in a different direction (Figure 1C). Beginning of the periodontal membrane and alveolar bone region drew attention to the ongoing increase in osteoclastic cells. Nicotine can be applied to another section of the group, gingival epithelium cells hypertrophy and an increase in mitotic activity was observed. Mononuclear cell infiltration of the gingival connective tissue junction, and a thickening of collagen fiber bundles were seen.

These results showed that the morphological and

histological nicotine 2 mg/g administration fibrous periodontal membrane and alveolar bone disorder is caused by degeneration of the cells. pulp tissue, vimentin expression was intense in the cells of mesenchymal origin.

Cytokeratin change with the change of shape due to cell degeneration were observed (Figure 1D). The group treated with nicotine, cement and vimentin expression was observed in the periodontal membrane density irregularities along the collagen fibers were observed (Figure 1E). Mesenchymal tissues that is stained positive for vimentin lay underneath the epithelium. A strong expression of vimentin can be observed in formed periodontal ligament; negative expression of vimentin was observed in alveolar bone osteoblasts (Figure 1F).

## DISCUSSION

Nicotine is a major component of tobacco smoke. Tobacco smoking is probably the most important periodontitis, auditable environmental risk factor, which is shown to cause inflammatory changes (Palmer & Scott, 1999). Typically for cells of mesenchymal origin, periodontal ligament fibroblasts labelled for vimentin. By analogy with other tissues, the accumulation of vimentin may be related to mechanical loading. There is evidence to suggest that it accumulates in cartilage and fibrocartilage and endothelial cells in response to mechanical loading (Ralphs *et al.*, 1992; Schnittler *et al.*, 1993; Benjamin *et al.*, 1994). In rats, daily administration of nicotine adversely affects the periodontal tissues in a dose-dependent manner. Nicotine bone-forming cells and cellular proliferation and collagen synthesis (Lenz *et al.*, 1990). This is caused by inadequacies in the dentin matrix, odontoblast function can be expressed in danger.

*In vitro* studies have shown nicotine negatively affected osteoblasts (Yuhara *et al.*), inhibited gingival fibroblast growth and production of fibronectin and collagen, while promoting collagen breakdown (Tripton & Dabbous, 1995). It also affects periodontal ligament fibroblasts (Pinto *et al.*, 2002) and stimulates osteoclasts activity (Yuhara *et al.*; Henemyre *et al.*, 2003). The group treated with nicotine, cement and vimentin expression was observed in the periodontal membrane density irregularities along the collagen fibers were observed. Nicotine, soft tissues such as gingiva and periodontal ligament fibroblasts growing vascular congestion and a negative impact in terms of activity. As a result, the ability of nicotine change the fibroblast metabolism by affecting the synthesis of extracellular matrix molecules could imagine.

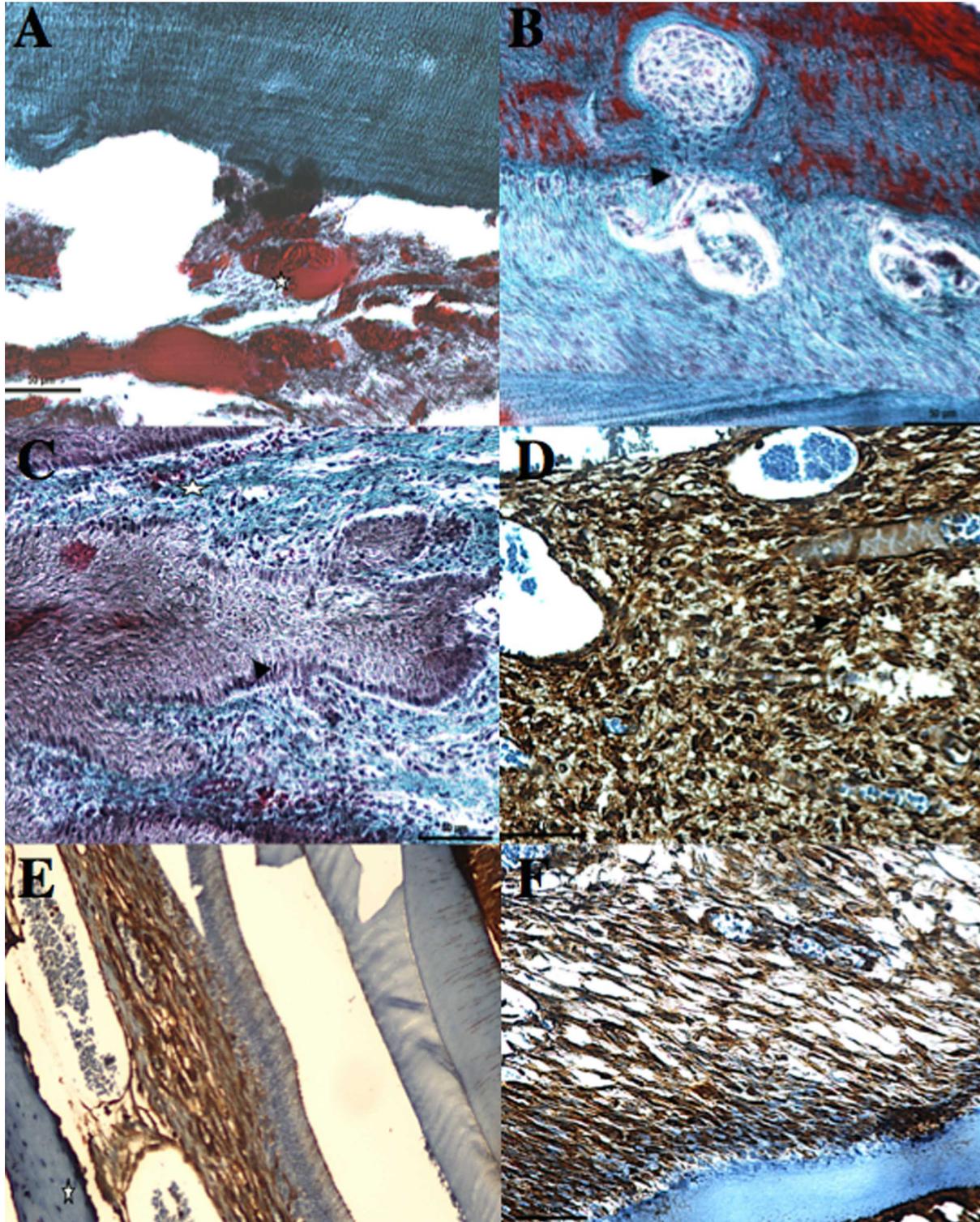


Fig. 1. A). Thinning of the collagen fibers in the pulp tissue, vascular congestion (\*) and inflammatory cell infiltration (Masson Bar 50 $\mu$ m). B). Dilatation of blood vessels and free erythrocytes, infiltration in perivascular area (arrow), degenerative cells in periodontal membrane and alveolar bone (\*) (Masson Bar 50 $\mu$ m). C). Hypertrophy in gingival epithelium and an increase in mitotic activity (arrow). Mononuclear cell infiltration of the gingival connective tissue junction (\*) (Masson Bar 50 $\mu$ m). D). An increase in the expression of vimentin in the pulp tissue (arrow) (Vimentin-Bar 50 $\mu$ m). E). Negative expression of vimentin in alveolar bone osteocytes (\*) (Vimentin-Bar 50 $\mu$ m). F). A strong expression of vimentin in periodontal ligament (Vimentin-Bar 50 $\mu$ m).

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**RESUMEN:** La nicotina es un alcaloide con efectos tóxicos sobre la cavidad oral. En este estudio, se utilizaron como animales de experimentación, 14 ratas Sprague-Dawley adultas con un peso entre 230-260 mg ( $\pm$  10 mg). Las ratas del grupo experimental (n = 6) fueron expuestas a nicotina sistémicamente con sulfato de nicotina, 2mg/kg vía subcutánea al día, durante 28 días. Fueron estudiadas la pulpa, tejido óseo alveolar, membrana periodontal y la unión del epitelio gingival en los dientes incisivos en secciones transversales y longitudinales. Se observó un adelgazamiento de las fibras de colágeno, congestión vascular e infiltración de células inflamatorias en el tejido de la pulpa. Los tejidos mesenquimales que tiñeron positivamente para vimentina se ubicaron debajo del epitelio. Una fuerte expresión de vimentina se pudo observar en el ligamento periodontal.

**PALABRAS CLAVE:** Nicotina; Rata; Vimentina.

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