

# Effects of Sildenafil on Dental Pulp: Immunohistochemical and Ultrastructural Evaluation

Efectos de Sildenafil en la Pulpa Dental: Evaluación Immunohistoquímica y Ultraestructural

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**SUMMARY:** Sildenafil is a strong peripheral vasodilator and is used to treat cardiovascular and neurosurgery. The purpose of this study was to investigate the immunohistochemical and ultrastructural effects of sildenafil on dental pulp of rats. The study was performed with adult female Wistar-Albino rats. Control group (n= 7) were fed on standard laboratory diet until surgery. The study group (n= 7) were administered sildenafil orally with orogastric tube 10 mg·kg<sup>-1</sup> once a day for 30 days. Each rat was anesthetized and incisor teeth were removed. This study examined the immunohistochemical and ultrastructural effects of sildenafil on the dental pulp in rats. The relaxation from the vessel, endothelial cell hyperplasia, moderate degeneration of collagen fibers were observed to cause degenerative changes in odontoblast with sildenafil. In the pulp tissue long-term use sildenafil is thought to cause degeneration and new vessel formation.

**KEY WORDS:** Sildenafil; Dental pulp; Morphological changes; Immunohistochemical study; Ultrastructural study.

## INTRODUCTION

Sildenafil is known as a selective and potent inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase-5 (PDE-5). PDE-5 catalyzes the hydrolysis of cGMP. Inhibition of PDE-5 causes increased concentration of cGMP and cyclic adenosine monophosphate (cAMP) (Bella *et al.*, 2007). PDE5 inhibitors are the treatment of erectile dysfunction [sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis), udenafil (Zydena)] and idiopathic pulmonary hypertension [sildenafil (Revatio)], although several other potentials have also been identified, such as memory improvement, anticancer therapy and treatment of heart diseases (Glossmann *et al.*, 1999). The effect is seen as leading to vasodilation by increasing cyclic Guanosine Monophosphate (cGMP) by means of Nitric Oxide (NO) (Langtry & Markham, 1999; Robson *et al.*, 2004; Salcido, 2008; Sarifakioglu *et al.*, 2004). The effect on bone healing is an interesting current topic and there are studies showing benefits in the treatment of fractures (Akgül & Alemdaroglu, 2008).

The regulation of angiogenesis and collateral vascular formation is a complex process that involves stimulators,

inhibitors and modulators. Most angiogenic factors bind to specific receptors on the endothelial cells and induce basement membrane breakdown, endothelial cell migration and proliferation. In angiogenesis, several cytokines play important roles, but the vascular endothelial growth factor (VEGF) is considered to be vital (Arras *et al.*, 1998). VEGF binds to receptors on the endothelial cells, which results in their growth, proliferation, and migration (Ferrara, 1999).

The aim of this study was to investigate the effects of sildenafil pulp tissue with immunohistochemical and ultrastructural methods.

## MATERIAL AND METHOD

In literature, the reported dose of sildenafil to be administered varies from 3–20 mg·kg<sup>-1</sup> per day in animal models. A dose of 10 mg·kg<sup>-1</sup> sildenafil achieves possible systemic protective effects in rats. Higher doses (over 20 mg·kg<sup>-1</sup>) may have greater vasodilatory effects but they may

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also have side effects such as hypotension, diminished tissue perfusion and excessive anti-inflammatory response whereas doses  $<10 \text{ mg}\cdot\text{kg}^{-1}$  are less or not effective at all (Irkorucu *et al.*, 2008, 2009). For this reason, we chose to administer sildenafil as a single daily dose of  $10 \text{ mg}\cdot\text{kg}^{-1}$ . Rat incisor teeth were placed in 10 % formaldehyde solution for 2 weeks then fixed in Bouin's solution for 2 days. The fixed bone samples were decalcified in a 10 % acetic acid, 0.85 % NaCl and 10 % formalin solution. Paraffin blocks were then prepared in a standard manner. The four sections were taken 6–7 mm in thickness at 20 mm intervals from the paraffin blocks. These sections were stained with haematoxylin eosine and evaluated separately.

### Immunohistochemical Analysis

**VEGF Immunohistochemistry Stain.** Antigen retrieval process was performed twice in citrate buffer solution (pH 6.0); the first for 7 min, and later for 5 min, boiled in microwave oven at 700 W. They were allowed to cool to room temperature for 30 min and washed twice in distilled water for 5 min. Endogenous peroxidase activity was blocked in 0.1 % hydrogen peroxide for 20 min. Ultra V block (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 10 min prior to the application of primary antibodies (vWF antibody, rabbit-anti-vWF, 1/800, ab6994, Abcam) overnight. Secondary antibody (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 min. Slides were then exposed to streptavidin-peroxidase for 20 min. As a chromogen, diaminobenzidine (DAB Invitrogen, Carlsbad, CA, USA) was used. Control slides were prepared as mentioned above but with omitting the primary antibodies. After counterstaining with hematoxylin and washing in tap water for 8 min and in distilled water for 10 min, the slides were mounted with Entellan.

**Electron Microscopy Technique.** The pieces of tissue were immediately placed in 2.5 % glutaraldehyde, buffered for 4 h, then fixed in  $\text{OsO}_4$  for 2 h, dehydrated in graded ethanol and embedded in araldite. Semi thin sections of  $1 \mu\text{m}$  thick were cut and stained with methylene blue azure II for light microscopic examination. Thin sections of 70 nm thick were stained with lead citrate-uranyl acetate and examined and photographed under Karl Zeiss Evo LS10 Electron microscope.

### RESULTS

In the examination histologic of control group no change was determined in the structure of the dental pulp. Normal dental represented by connective tissue, with plenty

of collagen fibers and fibroblast cells (Fig. 1a). Histological analysis of the groups treated with sildenafil; degeneration of the odontoblast cells, dilatation of blood vessels and congestion also increase in collagen fibers in connective tissue was observed. Depending on the dilatation of blood vessels, thinning and separation in the basal membrane, also showed an increase in inflammatory cell infiltration (Fig. 1b).

**Immunohistochemical examination.** Superficial zone of dental pulp: fibroblasts and endothelial cells were positive (Fig. 1c). In the experimental group, the thin vascular wall endothelial cells and connective tissue fibroblast cells showed weak expression. However, VEGF showed positive reaction for degenerative odontoblast cells in the pulp dentin border (Fig. 1d).

**Ultrastructural examination.** In control pulp, subodontoblastic layer of the capillary was found to be located close to the odontoblast. Collagen fiber bundles could be seen at the periphery of some fibroblasts of the control group. Many mitochondria and secretory vesicles showed in odontoblasts. No inflammatory cells except a few macrophage were observed in the pulp tissue. Many fibroblasts and undifferentiated mesenchymal cells were identified (Fig. 1e). Ultrastructural analysis of the groups treated with sildenafil; Dilatation and congestion in the capillary, basal membrane thinning, endothelial cell hyperplasia, mild degeneration of collagen fibers of the connective tissue was observed. Also hyperplasia and vacuolar changes were observed in odontoblast cells (Fig. 1f).

### DISCUSSION

Sildenafil has significant effects on endothelial cell function and cellular apoptosis. Sildenafil has been shown to prolong erection in aged rats via AKT-dependent eNOS phosphorylation. Phosphorylation of eNOS is coincident with enzyme activation and increased eNO release (Zhang *et al.*, 2003). In rabbit models of cardiac ischaemia-reperfusion, sildenafil has been shown to reduce the area of ischaemia (Ockaili *et al.*, 2002). In the developing tooth, VEGF and VEGFR-2 are expressed in odontoblasts and the inner enamel epithelium and may regulate odontoblast development and the differentiation of inner enamel epithelium to ameloblasts (Aida *et al.*, 2005; Miwa *et al.*, 2008). VEGF expression in pulp fibroblasts and odontoblasts of human teeth is higher in immature than mature permanent teeth, suggesting a role of VEGF in tooth maturation (Wang *et al.*, 2007). Zhang *et al.* used sildenafil treatment ( $2 \text{ mg}/\text{kg} \cdot \text{p.o.}$ ) in a rat model of brain ischemia and found out that sildenafil promoted angiogenesis via VEGF. In our study,

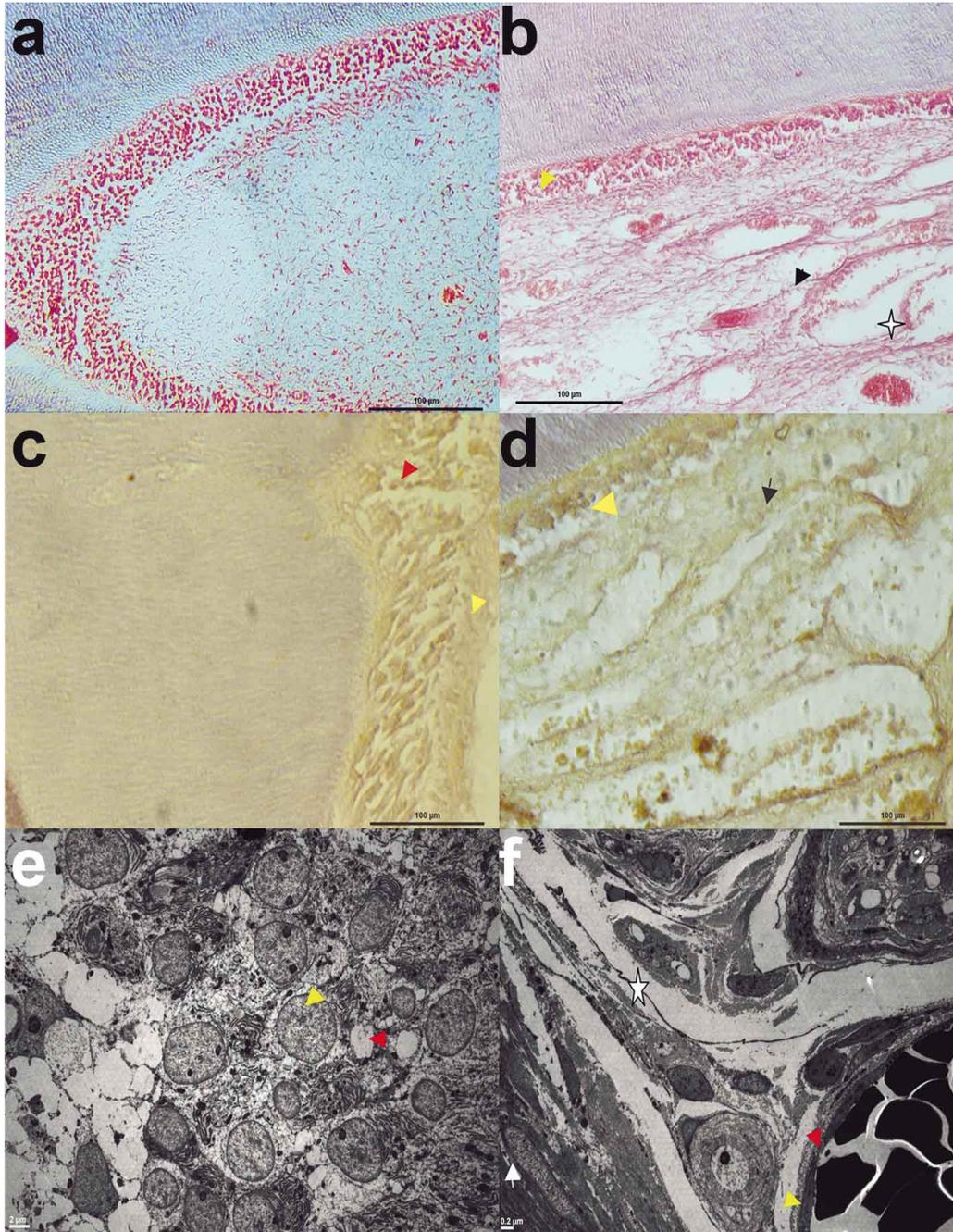


Fig. 1. a) Normal appearance of dental pulp (H-E staining, Bar 100  $\mu\text{m}$ ). b) Sildenafil group; Degeneration of the odontoblast cells (yellow arrow), dilation of blood vessels and congestion (star), thinning and separation in the basal membrane (arrow) (H-E staining, Bar 100  $\mu\text{m}$ ). c) VEGF expression in the control group by immunohistochemistry. VEGF expression of fibroblasts (yellow arrow) and endothelial cells (red arrow) in dental pulp (VEGF immun-staining, Bar 100  $\mu\text{m}$ ). d) Sildenafil group; Weak VEGF expression in fibroblast and endothelial cells (arrow); VEGF positive expression of degenerative odontoblast cells in the pulp dentin border (yellow arrow) (VEGF immun-staining, Bar 100  $\mu\text{m}$ ). e) In ultrastructural section of control group; Many mitochondria and secretory vesicles in odontoblasts (yellow), regular capillary vessels of subodontoblastic layer (red arrow) (Uranyl acetate and lead citrate staining, Bar 2  $\mu\text{m}$ ). f) Dilatation and congestion in the capillary, basal membrane thinning (red arrow), endothelial cell hyperplasia (yellow arrow), mild degeneration of collagen fibers of the connective tissue (star), hyperplasia and vacuolar changes in odontoblast cells (light arrow) (Uranyl acetate and lead citrate staining, Bar 0.2  $\mu\text{m}$ ).

the pulp tissue of rat treated sildenafil, increase blood vessel dilation also result in a reduction in VEGF expression has triggered the formation of new blood vessels.

For Yaman *et al.* (2011), sildenafil without causing any changes to the application of alveolar bone and gingiva pulp underline that caused the increase vascularity. Also gingiva flap surgery, gingiva injury, dental pulp, or is stated to be subject to the terms of sildenafil may be useful effects such as dental trauma. In this study, the thin vascular wall endothelial cells and connective tissue fibroblast cells showed weak expression. However, VEGF showed positive reaction for degenerative odontoblast cells in the pulp dentin border. Due to vascular dilatation, degenerative effect on endothelial cell hyperplasia and odontoblasts, also reduction in VEGF protein induced the formation of new vessels. Ultrastructural examination of the pulp tissue of sildenafil group; Dilatation and congestion in the capillary, basal membrane thinning, endothelial cell hyperplasia, mild degeneration of collagen fibers of the connective tissue was observed. Also hyperplasia and vacuolar changes were observed in odontoblast cells.

As a result, sildenafil, relaxation from the vessel, endothelial cell hyperplasia and moderate degeneration of collagen fibers were observed to cause degenerative changes in odontoblast. In the pulp tissue long-term use sildenafil is thought to be caused degeneration and new vessel formation.

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**SÖKER, S.; DEVECI, E.; YAMAN, F.; ATILGAN, S.; IPEK, F. & UYSAL, I.** Efectos del sildenafil en la pulpa dental: Evaluación inmunohistoquímica y ultraestructural. *Int. J. Morphol.*, 34(2):570-574, 2016.

**RESUMEN:** El sildenafil es un vasodilatador periférico importante y se utiliza para tratar enfermedades cardiovasculares y en neurocirugía. El propósito de este estudio fue investigar los efectos inmunohistoquímicos y ultraestructurales del sildenafil sobre la pulpa dental de ratas. El estudio se realizó con ratas Wistar albinas, hembras adultas. El grupo de control (n= 7) fue alimentado con una dieta estándar de laboratorio hasta que se realizó la cirugía. El grupo de estudio (n= 7) fue tratado con sildenafil por vía oral y sonda orogástrica 10 mg·kg<sup>-1</sup> una vez al día durante 30 días. Cada rata fue anestesiada y se extrajeron los dientes incisivos. Se examinaron los efectos inmunohistoquímicos y ultraestructurales del sildenafil sobre la pulpa dentaria. Con la administración de sildenafil se observó la relajación de los vasos, la hiperplasia de las células endoteliales y una degeneración moderada de fibras colágenas causando cambios degenerativos en los odontoblastos. En el tejido pulpar, el uso de sildenafil a largo plazo puede causar la degeneración y neoformación de vasos.

**PALABRAS CLAVE:** Sildenafil; Pulpa dental; Cambios morfológicos; Estudio inmunohistoquímico; Estudio ultraestructural.

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