

**Ministry of Higher Education  
& Scientific Research  
University of Baghdad  
College of Dentistry**



# **Histological and immunohistochemical (Annexin A1) Analysis of Bisphosphonate (Zoledronate) Effect on Submandibular and Parotid Glands in Neonatal Rats**

A Thesis

Submitted to the council of the College of  
Dentistry/University of Baghdad in partial fulfillment of the  
requirements for the degree of Master of Science in Oral  
Histology

By

**Issa Hasan Issa**  
B.D.S.

Supervised by

Assistant Professor

**Dr. Nada M.H. Al-Ghaban**

B.D.S., M.Sc., Ph.D. (Oral Histology and Biology)

**2017 A.D.**

**1438 A.H.**

# Abstract

**Background:**

Zoledronate (zometa), a third generation of bisphosphonate, is used for the treatment of bone metastases in multiple myeloma or solid mass and for hyper-calcaemia of malignancy. Although, the way of action of zometa is chiefly studied in bone, few are known about its effects on the salivary gland. Annexin a1 contributes to a wide range of cellular biological activities, including anti-inflammatory effects, inhibition of cell proliferation and cell death regulation.

**Aim of the study:**

To assess the effect of zoledronate on the general body weight as well as to evaluate the effect of administration of zoledronate on submandibular and parotid glands in newly born rats histologically and immunohistochemically on Annexin a1 expression. Lastly to compare the effect of zoledronate on parotid and sub mandibular glands on those rats.

**Material and method:**

The present study includes 40 newborn Wistar albino rats of both male and female sexes with average weight (40-60) gm, which were included in this study. The animals were randomly divided into four groups: the control group of 14 days sacrificed after injection (group A), the control group of 28 days sacrificed after injection (group B), the zoledronic acid -treated group of 14 days sacrificed after injection (group C) and the zoledronic acid -treated group of 28 days sacrificed after injection (group D).

Twenty rats, 10 from group A and 10 from group B, were injected with a single dose 0.1mg/kg of sterile saline solution intravenously through the tail vein at age of two weeks. The other twenty rats, 10 from group C and 10 from group D, were injected with a single dose of 0.1mg/kg of zoledronic acid intravenously directly through the tail vein at age of two weeks. The weight of

the animals was evaluated pre-injection and at the sacrificing day (post injection).

The animals were sacrificed by given them high dose of general anesthesia. Then each rat was washed with water then putted on the dissection pad. After dissection, the salivary glands are exposed, excised and collected in 10% formalin then blocked and processed to section. The sections were studied by (H&E) stain and immunohistochemical study for annexin a1 expression (ANXA1 antibody).

**Results:**

The result of this study illustrated a significant weight differences between experimental group and control group especially in 28 days after injections.

The histological results of this study revealed a relative increment in the secretory granules in the serous cells of the experimental groups over that of the control groups.

The immunohistological results revealed strong positive expression of ANXA1 in the ductal and myoepithelial cells of parotid and submandibular glands with high significant differences between experimental and control groups especially in 28 days after injection. In addition, weak positive immune-staining was found in many cells of the mucous acini of submandibular gland.

**Conclusion:**

Zolendronate may affect the secretory mechanism of parotid and submandibular glands due to either slowing exocytosis or incrementing the protein synthesis. The positive localization of annexin a1 in the ductal epithelium of salivary organs may be due to functional exchange of ions and proteins.