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# Evaluation of ethanolamine oleate sclerotherapy on the submandibular glands of canines as a potential therapy for sialorrhea

F. Abbas [a], R. Farid [b], I. Nada [a], M. El-Sharnouby [c,*]

[a] ENT Department, Faculty of Medicine, Misr University for Science and Technology, 6th of October city, Giza, Egypt
[b] Department of Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[c] ENT Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

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ABSTRACT

*Background:* Sialorrhea can have major negative effects on the physical and social well-being. Sclerotherapy may be useful in patients with sialorrhea by decreasing the amount of saliva production. The aim of this study was to test the effect of ethanolamine oleate (EO) in an experimental model as a preliminary step for its application in humans.
*Methods:* Histopathological and morphometric analysis of submandibular glands from thirteen dogs was preformed. A total of 25 glands were injected with 1 ml of 2.5% EO ($n = 5$), 1 ml of 5% EO ($n = 5$), 5 ml of 2.5% EO ($n = 5$) and 5 ml of 5% EO ($n = 5$). Five glands were used as control.
*Results:* EO significantly induced a dose dependent scaring of the gland ending in lobular transformation (salivary gland cirrhosis). Morphometric measurements showed that 1 ml of 2.5% or 5% EO significantly induced fibrosis compared to normal glands ($p = 0.014$ and 0.021, respectively). Fibrosis significantly increased and was more apparent when a dose of 5 ml of 2.5% EO or 5% EO were injected [by semi-quantitative evaluation ($p = 0.016$ and 0.002, respectively) and morphometric measurements ($p = 0.016$ and 0.008, respectively)]. This scarring effect was significantly associated with reduction of area of acinar cells when a dose of 1 ml–5%, 5 ml–2.5% or 5 ml–5% EO were applied ($p = 0.03$. 0.012 and 0.004, respectively). Moreover, ductal injury was only significant when a dose of 5 ml of 5% EO was used ($p = 0.034$). This dose and concentration (i.e. 5 ml–5% EO) had a significant synergetic effect [$p = 0.0119$].
*Conclusion:* In this model, treatment with EO proved to permanently reduce the acinar area through induction of progressive, irreversible and dose dependant scarring (medical sialoadenectomy).


## 1. Introduction

At rest a healthy person produces from 0.75 to 1.5 liters of saliva per day with the submandibular glands contributing to about 70% of saliva [1]. Sialorrhea, "drooling", may be due to excess salivation or defective swallowing. The latter can be due to lip incontinence, decreased oral sensation, defective oral stage of swallowing, an open bite and/or poor posture and neck flexion [1].

Drooling can have major negative effects on patients' physical and social well-being, predisposing to self-consciousness, social embarrassment, isolation, and depression [2].

Different treatment options exist. They include behavioral and biofeedback therapy, such as programs designed to improve body position and posture as well as oral motor skills[3]. Pharmacotherapy is another option, using anticholinergic drugs or skin patches [2,3]. Local injection of Botox [4] is a third modality. Surgical options include relocation of submandibular ducts, ligation of parotid ducts, chorda tympani neurectomy or excision of submandibular and parotid glands [2].

Botox, a neurotoxin which competes with acetylcholine for nerve-ending receptors, is gaining wide spread recognition in treating sialorrhea by leading to neurosecretory paralysis [4]. It is more effective and less time consuming than behavioral therapy; has less systemic side effects than anticholinergic medications; and does not carry risks of surgery. However, it is a short acting, yet expensive, treatment which often requires repeated injections [4].

Thus, another injectable material which could decrease saliva production, has a permanent effect and is inexpressive would be appealing. Ethanolamine oleate (EO) is a salt of unsaturated fatty acid which induces local sclerosis [5]. It has been successfully used as a sclerotherapeutic agent for the treatment of various pathologies [6–11].

The aim of this study was to evaluate the potential effect of EO injection as a sclerosing material on the substance of the submandibular salivary gland. Ethanolamine oleate was chosen due to its potency, safety, ease of application and availability [11].

* Corresponding author at: 8 Mokhtar ElMasry Street, Ard Elgolf, Nasr city, Cairo, Egypt. Tel.: +20 1287187654.
*E-mail address:* mmsharnouby@yahoo.com (M. El-Sharnouby).

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## 2. Material and methods

After approval of the department review board of ENT department, MUST University, a blinded randomized controlled animal trial was conducted. Thirteen healthy stray dogs from Veterinary school of medicine, Cairo University, were randomly selected. All dogs were of no specific type or bread and were from both genders. Initial aspiration was always done to avoid inadvertent injection into a blood vessel. The dogs were randomly divided into three groups.

- **Group I (n = 5 dogs):** left submandibular glands ($n$ = 5) injected with 1 ml of 2.5% EO (1 ml–2.5%), right submandibular glands (n = 5) injected with 1 ml of 5% EO (1 ml–5%).
- **Group II (n = 5 dogs):** left submandibular glands ($n$ = 5) injected with 5 ml of 2.5% EO (5 ml–2.5%), right submandibular glands ($n$ = 5) injected with 5 ml of 5% EO (5 ml–5%).
- **Group III (n = 3 dogs).** Five submandibular gland were used as control ($n$ = 5).

Bilateral sialoadenectomy was done after 6 months. The anesthesia and surgical work were consistent with the Australian national health and medical research council (NHMRC) guidelines on the care of dogs used for scientific purposes [12]. A total of 25 glands were analyzed.

### 2.1. General histological procedures

Submandibular glands were fixed in 10% formalin solution. Sections were routinely stained with haematoxylin-eosin for general morphology and Masson's trichrome stain to identify collagen fibers. Histological findings were interpreted in reference to Dreyer et al. [13]. The pathologist was blinded about the specimen groups.

### 2.2. Semi-quantitative histological evaluation:

Semi-quantitative histological evaluation was adapted from Dreyer et al. [13], to evaluate degree of fibrosis, inflammation and acinar loss.

*Fibrosis:* 0 = absent (normal histology), 1 = mild focal expansion, 2 = moderate diffuse expansion, 3 = severe expansion without lobular transformation, 4 = lobular transformation (salivary gland cirrhosis). *Inflammation:* 0 = absent, 1 = mild, 2 = moderate, 3 = severe. *Acinar injury:* 0 = absent, 1 = ectatic ducts, 2 = ectatic ducts and duct proliferation, 3 = acinar loss.

### 2.3. Morphometric methods

Morphometric analysis of fibrosis was done according to Souza et al. [14]. The area of the fibrous tissue in 10 selected microscopic fields, blue stained, was directly measured and calculated as the medium percent of the total area examined. The medium acinus area in 10 selected microscopic fields for each gland was similarly measured. Morphometric measurements were analyzed by Computerized Image Analyzing Software (Special FIF starter. version 3.2, Olympus, Germany).

### 2.4. Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2010. The results were expressed as mean ± SD. For comparison between the control and group I &II data, unpaired tier 2 $T$-test was performed. $p$ value $<0.05$ was considered statistically significant. The results of the study of the three groups were analyzed statistically using the analysis of the variance (ANOVA).

## 3. Results

Injection of ethanolamine oleate (EO) did not induce local inflammation at the site of injection. Six months later, during sialadenectomy the glands were easily dissected. Grossly, no necrosis was noted and the capsule was intact.

Histologically, control submandibular glands showed a mixture of mucous and serous secretory acini. The acini contain few intercalated ducts with narrow lumen and larger striated ducts. Acini were separated by thin fibrous tissue septa containing nerves, vessels and interseptal ducts. There was no evidence of fibrosis, inflammation, or parenchymal injury (Figs. 1 and 2). Injection of EO induced variable degree of fibrosis ending in focal lobular transformation (salivary gland cirrhosis) (Fig. 1).

The effects of EO were judged by its histological effects (fibrosis, inflammation, signs of acinar injury) and by morphometric measurements of the percentage of fibrosis and the resulting acinar surface area. The effect of EO was dose dependent (Figs. 2 and 3). Injection of 1 ml of 2.5% or 5% EO induced mild degree of fibrosis in the glandular interstium. Compared to control glands, this fibrosis was insignificant by semi-quantitative histologic evaluation (Table 1) but significant with morphometric measurements (Table 2). On the other hand, application of 5 ml of 2.5% or 5% EO induced significant fibrotic effect by semi-quantitative evaluation (Table 1) and morphometric measurements (Table 2). The fibrotic effect of EO was accompanied by decrease in the acinar surface area (Table 2). Compared to the control glands, injection of

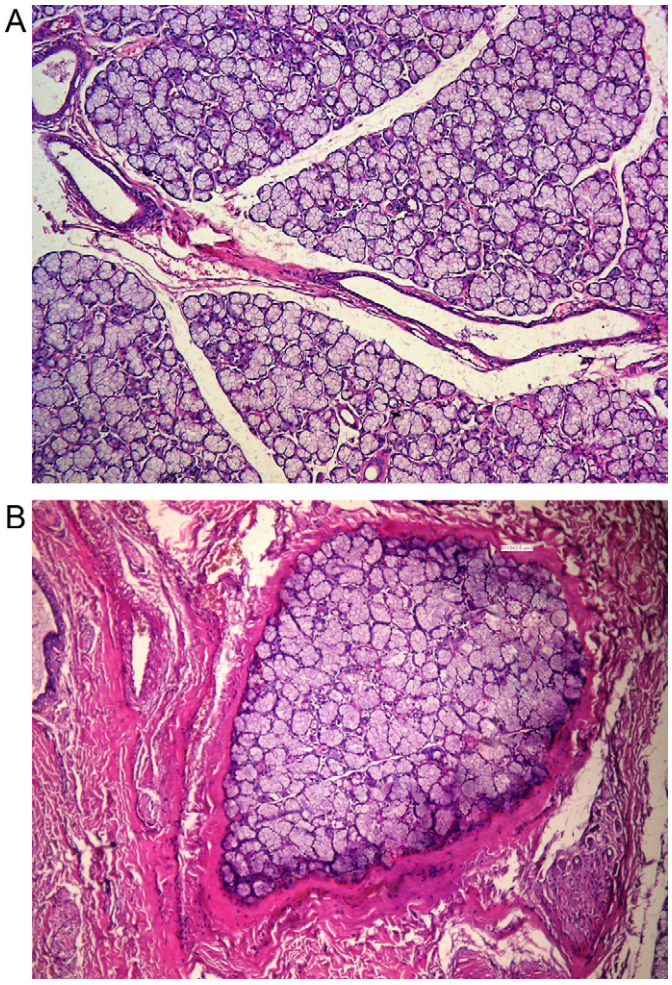


**Fig. 1.** Normal submandibular gland (A) and lobular transformation (salivary gland cirrhosis) (B) after injection of 5 ml–5% EO (H&E ×100).