

1. Introduction

Imidazoline derivatives (ImDs) such as oxymetazoline (OXY) and xylometazoline are highly effective hemostatic agents that act directly on alpha adrenergic receptors within the sympathetic nervous system (Fig.1). ImDs have been routinely used in medicine to treat ophthalmic inflammation, nasal congestion, and to control bleeding during otolaryngology surgery.^{1,2} The addition of Benzalkonium chloride (BKC) to ImDs has demonstrated a reduction in bacterial contamination.³ This **dual function** (hemostasis & antimicrobial activity) of **ImDs + BKC** illustrated in Fig.2 warrants additional research in dentistry to gain a better understanding of its pharmacological and mechanistic actions in pulpal management procedures such as a direct pulp cap, managing an exposure in a permanent tooth with an immature root (e.g. apexogenesis), and a primary tooth pulpotomy.³

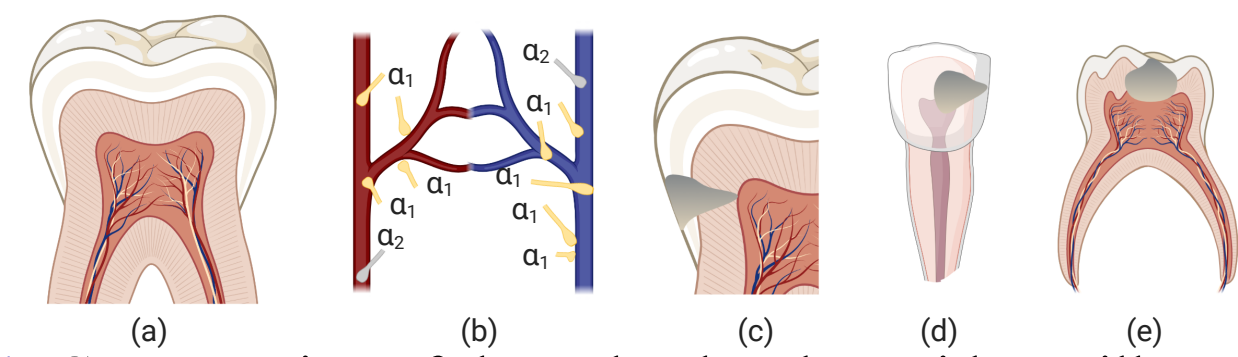


Fig.1: Cross-section of the pulp chamber with capillary plexus and post-capillary venules showing sympathetic innervation dominated by post synaptic α -1 adrenoreceptors. *

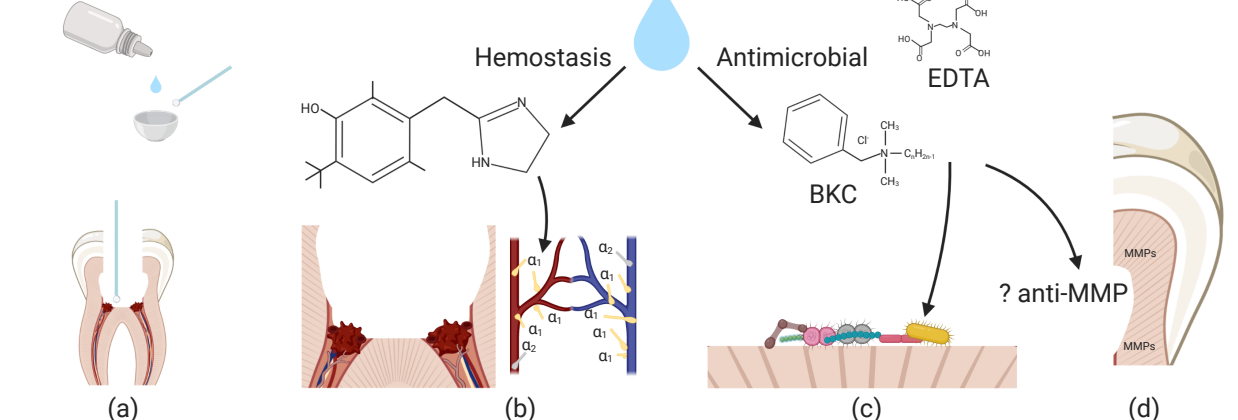


Fig.2: Conceptual Model of Imidazoline Activity showing dual function on application to exposed pulp chambers.*³

2. Purpose

This study evaluated the antibacterial properties of a potential pulpotomy medicament, Afrin (OXY-0.05% with Benzalkonium chloride (BKC), against two native oral bacterial species, *Streptococcus mutans* and *Neisseria mucosa*.

3. Material and methods

Using a disc diffusion antibiotic susceptibility test, *S.mutans* was grown on brain-heart infusion (BHI) agar plates (with 0.5% glucose) and exposed to OXY (0.05%), BKC (0.025%), Afrin (OXY-0.05% with BKC), Ferric sulfate (20%;ViscoStat), and deionized water (DI). The zones of inhibition (ZOI) were recorded after 20hr of incubation at 37 ± 0.5 °C under anaerobic conditions. Broth inhibition assay was conducted with *N.mucosa* and the data analyzed (OD & pH) over 20hr using 1-way ANOVA.

4. Results

1) Disc Diffusion Assay

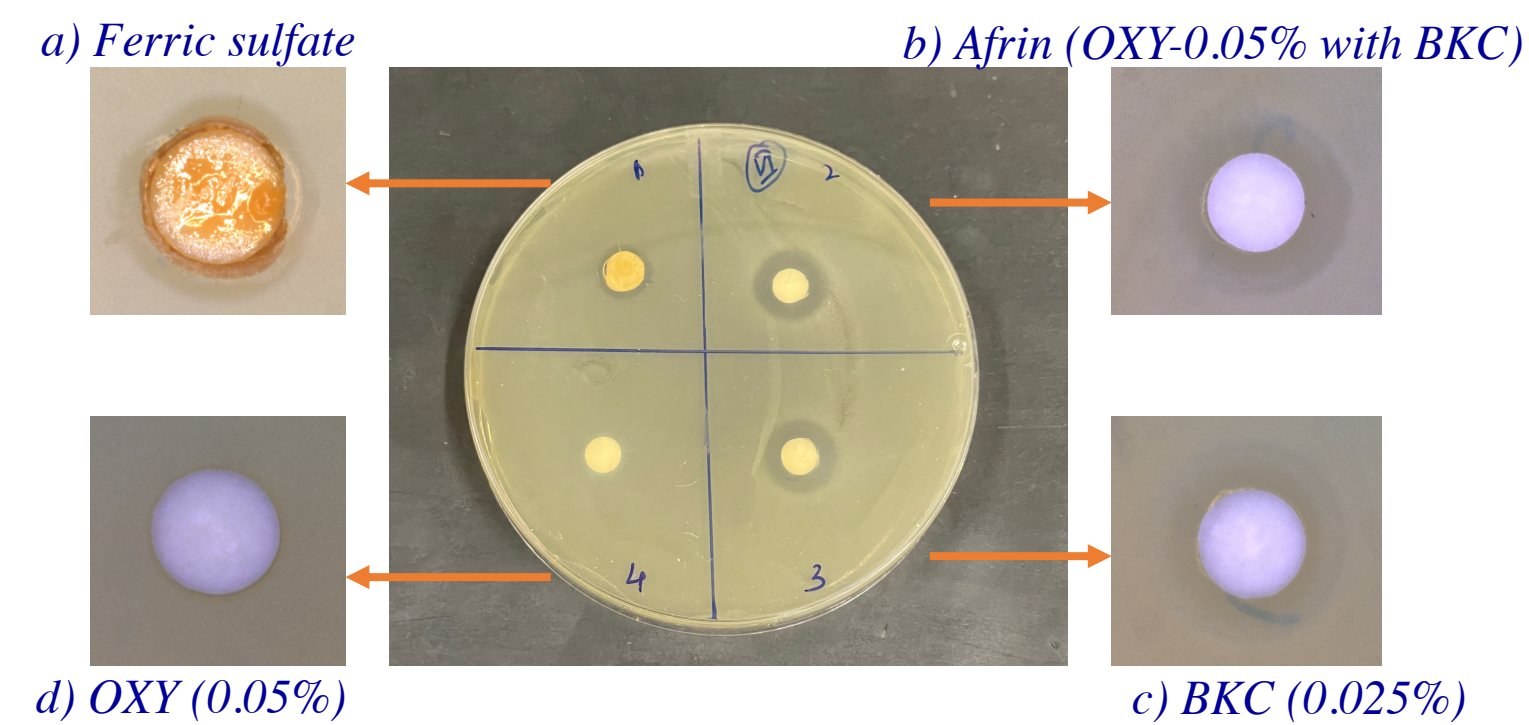


Fig.3: In the disc diffusion assay, 4 test samples were used. Afrin (b) and BKC (c) had the largest ZOI measuring 12.6 ± 0.8 and 12 ± 0.9 mm respectively, indicating antibacterial activity against *S.mutans*. Ferric sulfate (a) demonstrated a very small ZOI, while Oxymetazoline (positive control) had no ZOI.

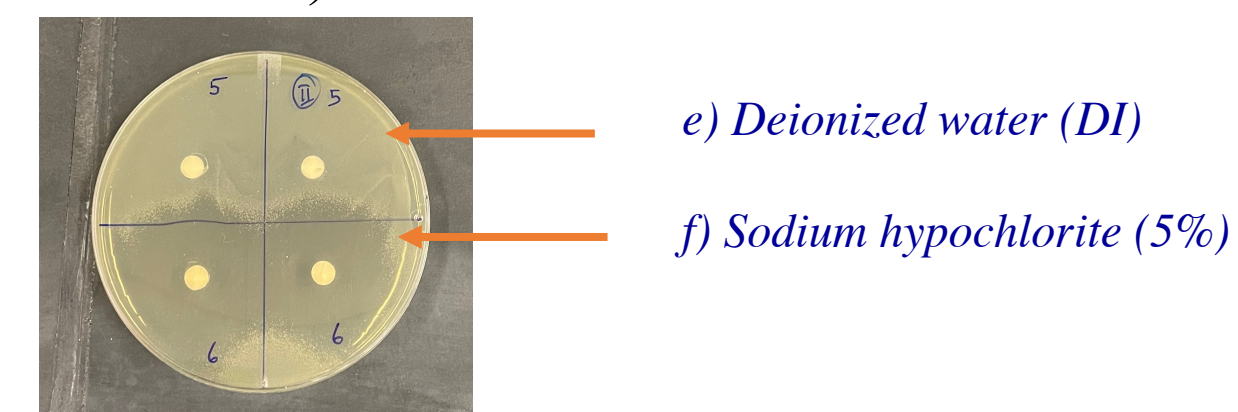


Fig.4: Additional controls were added to the study for comparison. Deionized water (e) (negative control) had no ZOI while sodium hypochlorite (5 % bleach) (f) displayed a distinct clear appearance spanning the entire quadrant of the agar plate (~40mm) indicating potent antimicrobial action.

4. Results

2) Zone of Inhibition (ZOI)

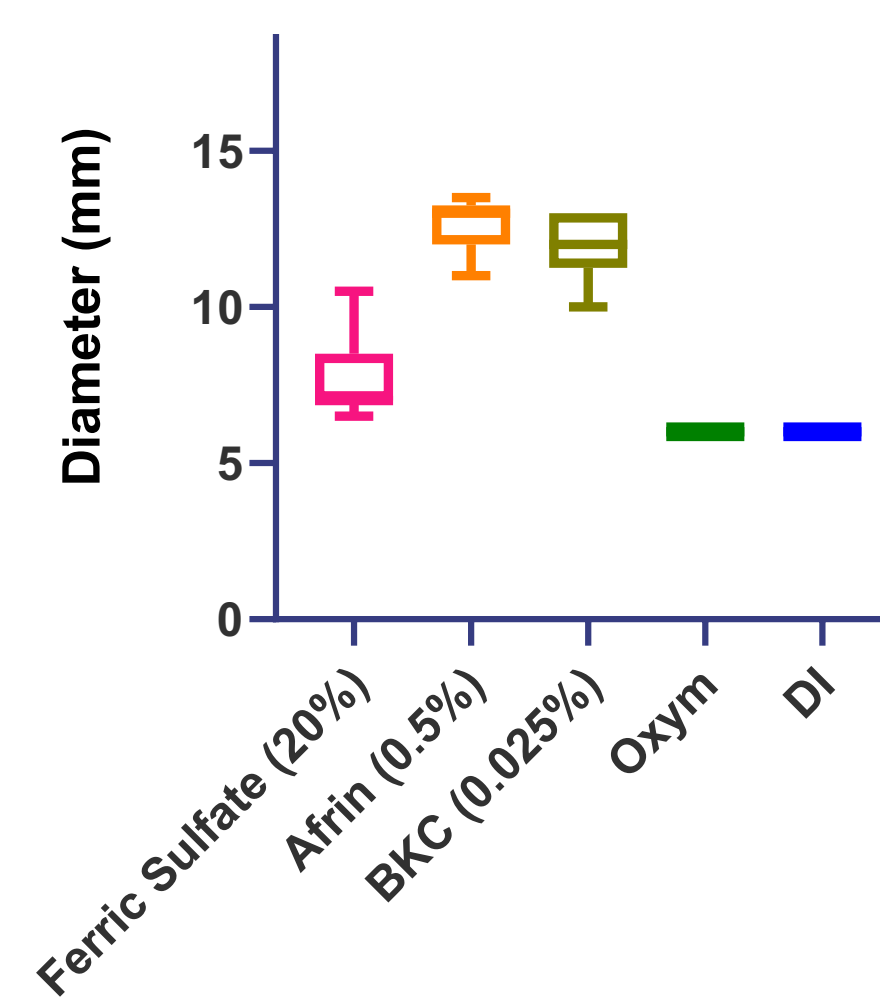


Fig.5 illustrates a box plot of the diameters and standard deviations of the ZOIs for each test sample against *S.mutans*. Among the materials tested, Afrin and BKC had the largest inhibition zones. 1-way ANOVA was used with Graphpad Prism and MS excel for statistical analysis of data ($n=13$; $p<0.05$).

3) Broth Inhibition: Measurement of OD and pH

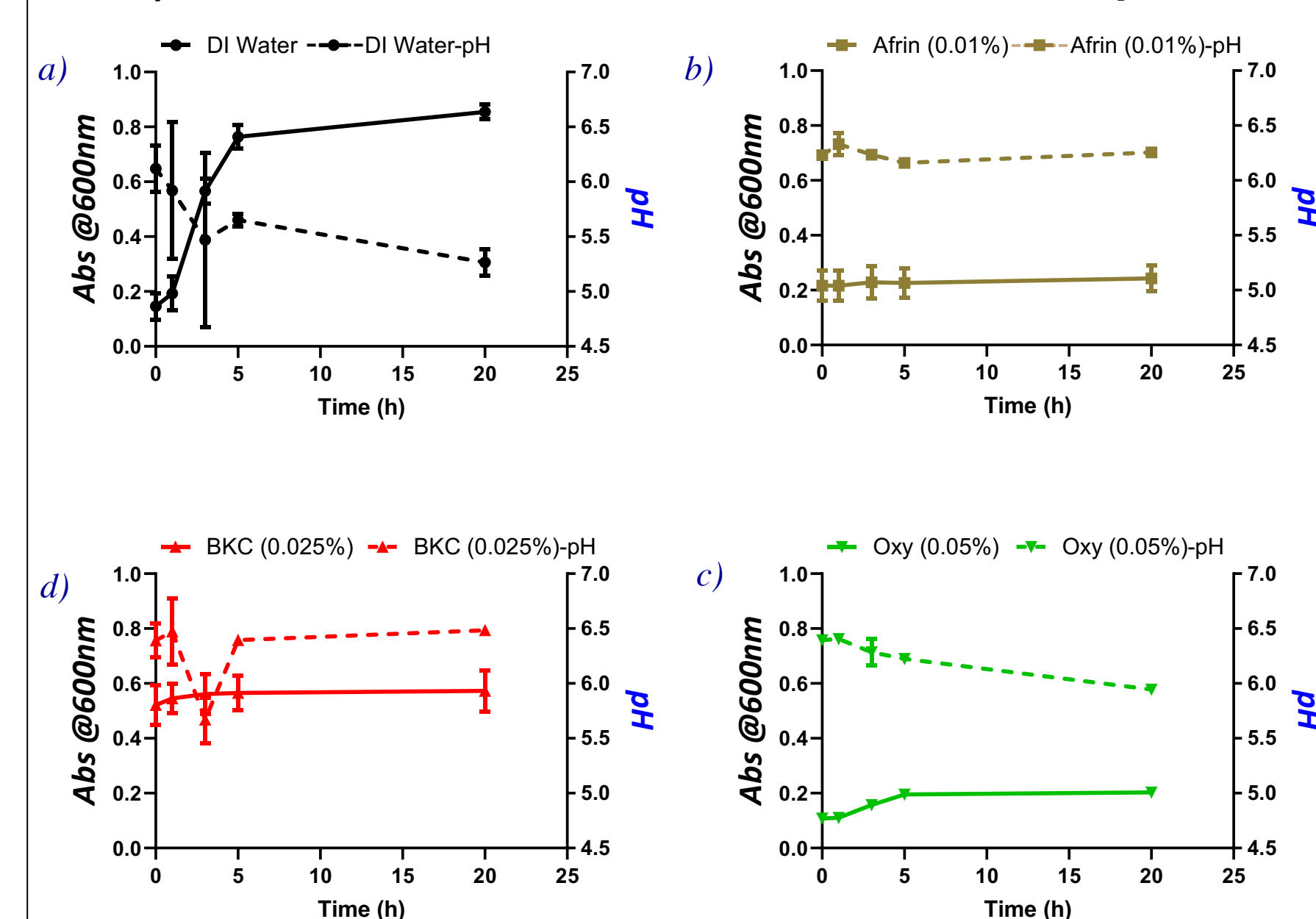


Fig.6: Broth inhibition test showed stable antibacterial effects against *N.mucosa*, OD and pH levels, for test samples containing Afrin (0.01%) (b) and BKC (0.025%) (d) for up to 20 hours indicating antimicrobial activity. DI (a) and Oxymetazoline (c) containing samples showed an elevation in OD indicating a rise in bacterial count and a concurrent drop in pH.

5. Discussion

- ImDs are used in bottles that have repeated nasal use and BKC is added to these bottles to reduce bacterial contamination. Use of these solutions (ImDs + BKC) for pulpal management can utilize this preservative for a **dual purpose** besides just hemostasis.
- Future studies need to be conducted to optimize the effective dose and volume of Afrin to establish pulpal hemostasis and antimicrobial activity.

6. Conclusion

Afrin (OXY-0.05% with BKC) demonstrated a significant antibacterial action, likely from the addition of BKC, against abundant oral bacteria that are found in pulpal infection (*S.mutans* and *N.mucosa*). Afrin's additional hemostatic properties may have important clinical implications in pulpal management of primary teeth.

7. References

- Cartabuke, R.S., Anderson, B.J., Elmaraghy, C., Rice, J., Tumin, D. and Tobias, J.D., 2019. Hemodynamic and pharmacokinetic analysis of oxymetazoline use during nasal surgery in children. *The Laryngoscope*, 129 (12), pp.2775-2781.
- Katz, R.I., Hovagim, A.R., Finkelstein, H.S., Grinberg, Y., Boccio, R.V. and Poppers, P.J., 1990. A comparison of cocaine, lidocaine with epinephrine, and oxymetazoline for prevention of epistaxis on nasotracheal intubation. *J. Clin. Anesth.*, 2(1), pp.16-20.
- Jones, R.S., 2021. Conceptual model for using imidazoline derivative solutions in pulpal management. *J. Clin. Med.*, 10(6), p.1212.
- * Fig.1 & 2 – designed in Biorender.com