Antimicrobial Effect Of Propolis And Propolis Containing Calcium Hydroxide And Zinc Oxide Eugenol Pastes On Microorganisms Located In Necrotic Primary Molars : A Comparative Study

A Research Proposal Submitted to the Faculty of Dentistry
Ain-Shams University in Partial Fulfillment of the
Requirements for the
Master Degree in Pediatric Dentistry.

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2016

Acknowledgement

*First of all, I would like to thank **ALLAH**, the most Merciful for assisting and directing me to the right way.*

***I** would like to express my deep gratitude and grateful appreciation **to, Dr. Noha Samir Kabil**, Associate Professor in Pediatric Dentistry And Dental Public Health Department, **Faculty** of **Oral and Dental Medicine, Ain Shams University**, who gave me the confidence and support to complete my Master's thesis issue. You challenged me **to look** for solutions. **I** have learned to believe in my future, my work and myself.*

*I would like to gratefully acknowledge the support, supervision and kindness of **Dr. Amira Saad Badran**, Lecturer in Pediatric Dentistry and Dental Public Health Department , **Faculty of Oral and Dental Medicine, Ain Shams University.** Her support and continuous guidance enabled me to begin and complete my work successfully and she gave me **generous** help throughout my study.*

*I would like also to express my greatful appreciation to **Dr. Nadia Mohamed El-Sheshtawy**, Lecturer of Microbiology &Immunology, **Faculty of Medecine**, **Ain Shams University** for her great help, support and guidance throughout my thesis studies.*

*Also I would like to thank **Dr. Ali Zaki** , Professor of Microbiology &Immunology , **Faculty of Medecine**, **Ain Shams University** for his great effort and help to complete my practical work.*

Finally endless thanks are extended to chairman, my professors, colleagues and staff members of Pediatric Dentistry and Dental public health department for their support and concern.

DEDICATION

I dedicate this thesis to my parents. Without their constant love and support, it would not have been possible for me to overcome the obstacles in my way and to achieve my dreams.

To the soul of my father, I wish you were here and I will never forget your words to me "I feel that you will be a good doctor"

To my mother, your great help and your prayers are the reasons of my success.

To my husband, you gave me all the support I needed to complete my work.

To my Sister & Brother, thank you for your support and encouragment during the years of work and personal life as well.

Finally to my little sweethearts, Asser & Hala , love you and sorry for every moment I was busy away from you.

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List of abbreviations	
Word	**Abbreviation**
Acute necrotizing ulcerative gingivitis	ANUG
Bacillus Subtilis	B.subtilis
Bacteroids melaninogenicus	B.melaninogenicus
Brain heart infusion	BHI
Calcium Hydroxide	$Ca(OH)_2$
Calcium hydroxide paste	CHP
Camphorated phenol	CP
Camphorated parachlorophenol	CPC
Camphorated paramonochlorophenol	CPMCP
Candida Albicans	C.albicans
Chlorhexidin	CHX
Colony forming unit	CFU
Dialister pneumosintes	D.pneumosintes
Dimethyl sulfoxide	DMSO
Enterococcus Faecalis	E.faecalis
Escherichia Coli	E. Coli
Ethanol extract of propolis	EEP
Fusobacterium nucleatum	F. nucleatum
Guedes-Pino paste	GPP
International Agency for Research on Cancer	IARC
Klebsiella pneumonia	K. pneumonia

Lentheen Broth	LB
Millimeters	mm
Minimum bactericidal concentration	MBC
Minimum inhibitory concentration	MIC
Paramonochlorophenol	PMCP
Parts per billion	PPB
Porphyromans gingivalis	P.gingivalis
Prevotella nigrescens	P.nigrescens
Pseudomonas aeruginosa	P.aeruginosa
Reduced transport fluid	RTF
Staphylococcus aureus	S.aureus
Streptococcus Faecalis	S. Faecalis
Streptococcus mutans	S. mutans
Streptococcus sanguis	S. sanguis
Tri-antibiotic mixture	TAM
Zinc oxide	ZO
Zinc oxide eugenol	ZOE
Zinc oxide Paste	ZOP
Zinc oxide eugenol paste	ZOEP

Introduction

The dental pulp is a unique tissue and is crucial in the long-term prognosis of the tooth. [1] Correct diagnosis and management of the pulpal status is mandatory as, when a carious lesion approximates the dental pulp, pulpitis occurs followed by pulp necrosis. If pulp necrosis is left untreated , this may lead to a localized abscess or a spreading infection into the surrounding soft tissue that ends up with cellulitis, which in some extreme cases can even be life threatening.[2]

The root canal infection is usually polymicrobial dominated by anaerobic bacteria that use tissue remnants and serum proteins as nutrients.[3]Aspirates from endodontic abscesses usually contain many different types of bacteria including; gram–positive anaerobic cocci, gram-negative anaerobic rods, facultative anaerobic streptococci, filaments, spirochetes and sometimes fungi.[4]

Several medications have been reported in the treatment of necrotic pulp including zinc oxide eugenol, calcium hydroxide, iodoform and camphorated parachlorophenol.[5]

Calcium Hydroxide is an excellent therapeutic option when used as an intracanal medication because of its reported antimicrobial action and its ability to stimulate mineralization. [6]

Zinc oxide eugenol has been the material of choice since 1930's.[7] However, it has certain disadvantages, therefore research is going on in this area to improve the properties of zinc oxide eugenol by altering it with other materials or by adding antibacterial substances.[8]

Calcium hydroxide and iodoform pastes showed high levels of biocompatibility when compared to other commonly used root canal filling materials. (9)

Nowadays there is a great trend to use natural materials as a cure for many diseases. A quote was posted by Albert Einstein, as follows“ Look back into the nature and then you will understand everything better.” This quote clearly points towards directing the use of the old tradition therapies with minimum side effects, like apitherapy, rather than the use of the more expensive antibiotic therapies. (10)

Propolis is a known natural material , that is used in the medical and dental fields as immune-modulatory agent, anti-inflammatory agent and antibiotic. (11) Some researches considered associating propolis with calcium hydroxide and zinc oxide eugenol, in order to add all the beneficial biological properties of propolis to those of calcium hydroxide and zinc oxide eugenol. These associations could aggregate the benefits of each material. (12,13)

Review of literature

The dental pulp is located in a rigid chamber, which makes it a unique tissue in providing strong mechanical support and protection from the microbes found in the oral cavity. [(1)] If this rigid shell loses its structural integrity, the pulp will get threatened by the adverse stimuli from the mouth, such as caries, cracks, fractures and open restoration margins, and thus all of these provide pathways for micro-organisms and their toxins to enter the pulp.[(14)]The pulp initially inflames in response to irritation and will become necrotic and infected if left untreated.[(15)]

Pulpally infected primary teeth must be retained until exfoliation whenever possible as the primary teeth are considered to be the best space maintainers, thus the main aim of root canal therapy in pediatric dentistry is the maintenance of primary teeth until the eruption of the permanent dentition under healthy conditions.[(16)]

Non vital root canal therapy in primary molar teeth is carried on when the criteria for a classical pulpotomy cannot be met. The success of any root canal therapy depends on the elimination or reduction of bacteria from within the root canal space. This is achieved by adequate root canal debridement, antimicrobial irrigation and obturation with an insoluble antimicrobial material.[(17)]

Pulp therapy in primary teeth is more challenging than that of permanent teeth, this is due to the complex anatomy of their root canal systems especially the great number of collateral canals, the presence of apical ramifications, molar root curvature, the physiological tooth resorption process and their proximity to the

developing permanent tooth, in addition to the difficulty in behavior management in children.[18]

Rationale for Root Canal Therapy in Primary Molars

Pulpectomy is indicated in primary teeth in which, the radicular pulp exhibits hyperemia, or evidence of necrosis of the radicular pulp, with or without carious involvement. [19]

Caution to carry out root canal treatment in primary molars may be needed due to the lack of consistent evidence based treatment protocol and medicaments, in addition to the difficulty associated with cleaning and shaping of the complex root canals of primary molars.[20,21]

Unlike permanent teeth, preparation of the root canals in primary molars , is based on the use of chemical agents and by the use of an antimicrobial root canal filling material rather than mechanical debridement. [22]

The advantage of root canal therapy is that it preserves masticatory function and maintains space for the succedaneous permanent tooth as extraction of primary teeth and the use of space maintenance to prevent a loss in arch length, acquires additional cost, appliance maintenance, oral hygiene care and more frequent recall visits. [23]

Other reasons for primary root canal therapy include preservation of a pulpally involved primary tooth in the absence of a succedaneous tooth, prevention of abnormal tongue habits, maintenance of esthetics, prevention of possible speech problems and prevention of possible psychological effects of premature tooth loss.[24]

Bacteria of pulpal disease

Bacteria involved in pulp disease first attach to the dentinal walls and colonize this surface, forming authentic biofilms. Pulpal inflammation results from the diffusion of bacterial products through dentinal tubules before the tissue is exposed by a long time. After exposure, the pulp tissue is in direct contact with bacteria and their products, leading to severe inflammation. [(25)] Bacteria in the battle front have to survive from the host defenses attacks and have to acquire nutrients at the same time to keep themselves alive. In this bacteria-pulp clash, the pulp invariably is defeated and becomes necrotic. Therefore, bacteria move forward and colonize the necrotic tissue and become the first root canal colonizers. [(26)]

A study was conducted out to evaluate the bacterial prevalence in thirty one root canals of necrotic human deciduous teeth with periapical lesions using bacterial culture. Crown access was performed then samples were collected using absorbent paper points for microbiological evaluation and determination of colony forming units (CFU). Aerobic microorganisms were found in 93.5% of the samples, anaerobic microorganisms in 96.7%, streptococci in 96.7%, Streptococcus mutans (S. mutans) in 48.4% and black-pigmented bacilli in 35.5%. It was concluded that in human necrotic deciduous teeth root canals, the infection is polymicrobial, with a large number of microorganisms and a predominance of streptococci and anaerobic microorganisms. [(27)]

In order to determine the microbes of primary and secondary infected root canals, a study was conducted in which microbial samples were taken from sixty root canals, forty one from primary infected necrotic pulp tissues and nineteen samples from secondary infected canals due to failed endodontic treatment. Strict anaerobic techniques were used for incubation and identification. Results showed that 224 cultivable isolates were identified belonging to fifty six different bacterial species. Individual root canals showed a maximum of ten bacterial species. Seventy percent (70%) of the bacterial isolates were either strict anaerobes or microphilic.

The anaerobes most frequently isolated were; Peptostreptococcus micros (35%),Fusobacterium nucleatum(F. nucleatum) (11.7%), Fusobacterium necrophorum (23.3%), Prevotella nigrescens (P. nigrescens) (16.7%), Porphyromonas endodontalis (5%) and Porphyromonas gingivalis (6.7%).

The root canal micro-organisms of untreated teeth with apical periodontitis was found to be mixed, comprising gram-positive and gram-negative and mostly anaerobic microorganisms and usually containing more than three species per canal. On the other hand, facultative anaerobic and gram-positive bacteria predominated in secondary infected canals, which harbored from one to two species per canal. [28]

Paulo et al [29] carried on another study in which a total of twenty deciduous teeth with necrotic pulp were selected. Crown access was performed followed by introduction of four sterile absorbent paper points sequentially into the root canal for collection of material. After