Cytotoxicity and flow rate of Triple Antibiotic Paste versus Propolis as an intracanal medicament.

(An In-Vitro study)

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قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

Dedication

To my Mother, *You are the reason for who I am today*

To my Father, *Thank you for encouraging me to always be a better person*

To my Husband, *My soul mate and my backbone who has been a rock of stability ,support and love throughout my life*

To my father in law, *Thank you for being my father, my mentor and my best friend*

To my Mother in law, *Thank you for always putting a smile on my face*

To my Daughter *you are my sunshine*

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TAP:Triple Antibiotic Paste

NAOCL: Sodium Hypochlorite

HPLF: Human periodontal ligament fibroblasts

CMCP: Paramonochlorophenol

CFU : Colony forming unit

$CaOH_2$: Calcium hydroxide

MIC: Minimum inhibitory concentration

DAP: Double Antibiotic paste

CAPE: Caffeic acid and Phenethyl ester

MBC: Minimum bactericidal concentration

EEP: Ethanolic Extract of propolis

TAM Triantibiotic mixture

FBS: Fetal bovine serum

CHX: Chlorohexidine

MBIC: Minimum biofilm inhibitory concentration

LDH: Lactate dehydrogenase leakage assay

E-MEM: Dulbecco's Modified Eagle Medium

DMSO: Dimethyl sulphoxide

IC50: Half maximal inhibitory concentration

EC50: Effective dose concentration

SRB: Sulforamide B assay

MTT:Microculture tertrazolium assay

ADA :American Dental Association

PBS: Phosphate buffered saline

HBSS: Hank's Balanced Salt Solution

Introduction

Elimination of microorganisms from infected root canal systems is challenging and important in success of root canal treatments. Hence, adequate cleaning; which is the most critical factor and shaping and establishing a coronal seal are the essential elements for a successful treatment.Disinfection of the root canal system depends on irrigants, rinses and inter-appointment medicaments.

Intracanal medicaments have been thought an essential step in killing the bacteria in root canal, it also aids in prevention of growth of resistant strains and removal the smear layer.It should be nonantigenic, nontoxic and noncarcinogenic. In addition, it should have no adverse effects on dentin or the sealing ability of filling materials. Furthermore, it should be relatively inexpensive, convenient to apply and cause no tooth discoloration. Other desirable properties for an ideal irrigant include the ability to dissolve pulp tissue and inactivate endotoxins.[1]

Several materials were used as **intracanal medicaments** such as antibiotics, calcium hydroxide, Chlorohexidine, steroid and formaldehyde based intracanal medications and recently phytotherapeutic agents such as propolis.The constant increase in

antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives which led to the introduction of phytotherapeutic agents, herbal and alternative medicine in dentistry.

Propolis, a very promising material used in herbal medicine, is collected by bees from plants or young shoots and barks of trees, it is a natural remedy that can be used to cure various diseases. Scientific research has revealed its antioxidant, antibacterial, anti-fungal, antiviral, anti-inflammatory, anti-tumor an immuno-modulating properties. It is dispensed in various forms, being a good antimicrobial and anti-inflammatory agent, can serve as a better intracanal irrigant and intracanal medicament. The toxic effect of this natural agent on the human and animal cells is still unclear and needs to be investigated.

Biocompatability and low cytotoxicty are essential requirements that the chosen intracanal medicament has to show. It was of great necessity to test the cytotoxicity of Propolis to determine whether it can be used safely as an intracanal medicament or not. Cytotoxic test include MTT assay which is a colorimetric assay for assessing cell viability. MTT is used to determine mitochondrial reductive function hence it is a good indicator of cell death or inhibition of growth.

MTT results usually indicate purple for viable cells (no cytotoxicity) and yellow for cell lysis (cytotoxicity).

IC_{50} test is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. It indicates the death of 50% of vital cells.

It is considered fundamental to pharmacological research and is used widely in drug comparisons to achieve accurate results.

In this study we focus on the cytotoxic effects of both propolis and Triple Antibiotic paste to evaluate the safety of Propolis paste clinical usage and whether it could potentially replace intracanal antibiotic therapy in the future.

Review of literature

Successful opportunistic infection in most parts of the human body usually require only the host defence mechanisms. However, endodontic infections require an alternative pathway due to the special anatomical challenges present in root canals, host defence that are able to eliminate infections in other places are unable to eliminate micro-organisms in root canals due to the difficult anatomical challenges therefor a combined effort is needed in host defence and treatment factors ,success in all treatment steps is required for complete elimination of endodontic microrganisms

The sequence of events in treatment of endodontic infections are complete instrumentation ,irrigation, systemic antibiotic therapy (if necessary) and intracanal medications between visits.

Intracanal medications complete the work done by instrumentation and irrigation to obtain a bacteria free root canal system. However, it ’s unclear the consensus of the use of intracanal medications ,their advantage and their ability to help obtain a bacteria free root canal system.

In this study we focus on the cytotoxic effect of triple antibiotic and propolis pastes when used as intracanal medications.

1: Intracanal medications :

Chong et al [2] discussed the role and advantages of placing intracanal medicaments in endodontic infections, he stated that intracanal medicament aid in eliminating bacteria, act as a barrier against leakage from temporary filling, help dry persistent wet canals, render canal content inert and help neutralise canal debris. He mentioned that most indications of the intracanal medicaments are rather questionable.

He stated that the role of intracanal medicament are secondary to thorough cleaning and shaping. Thorough canal instrumentation and debridement are emphasised and are more pertinent, He also mentioned that bacteriological sampling in cases that doesn't respond to treatment may be necessary to aid in the choice on the proper intracanal medicament .

Chong concluded that it is doubtful that routine interracial medicament is needed for the treatment of teeth with vital pulp. However, in extensive infections and in cases that require a long period of treatment we could benefit from the merits of intracanal medications in obtaining a bacteria free environment, but intracanal medicaments should be combined with an antibacterial irrigant and proper debridement, their role are considered rather secondary in treatment of infections.

He also concluded that temporary filling material should be prevent leakage.and analgesics aid in controlling pain better than intracanal