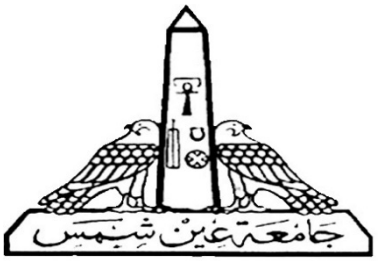

Faculty of dentistry Ain Shams University

Evaluation of Leptospermum Scoparium Honey as An Endodontic Intracanal Medicament

Thesis

Submitted to Endodontic Department Faculty of Dentistry,

Ain Shams University

For

Partial fulfillment of requirements for The Master Degree in Endodontics

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2018

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2018

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List of Abbreviations

Abbreviation	*Full term*
%	Percentage
*	Significant
$Ca(OH)_2$	Calcium hydroxide
TAP	Triple antibiotic paste
CFU	Colony forming units
CHX	Chlorhexidine
CMCP	Camphorated monoclorophenol
E. faecalis	*Enterococcus Faecalis*
MGO	Methylglyoxal
EDTA	Ethylene diamine-tetra-acetic acid
H_2O_2	Hydrogen peroxide
µ	micron
min	Minute
ml	milliliter
mm	millimeter
µm	Micrometer
µl	Microliter
$NaOCl_2$	Sodium hypochlorite
v/v	Volume per volume
ns	non-significant
UMF	Unique Manuka Factor
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MRSA	Methicillin resistant Staphylococcus aureus
MSSA	Methicillin sensitive Staphylococcus aureus
VRE	Vancomycin resistant Enterococcus
VSE	Vancomycin sensitive Enterococcus
AST	Antibiotic Sensitivity test
MHB	Mueller Hinton Broth
BHI	Brain Heart Infusion
P-value	Probability
°C	Degrees centigrade
h	Hour
s	Second
SD	Standard deviation
SEM	Scanning Electron Microscopy

Introduction

The success and failure of endodontic treatment relies largely on the complete eradication of bacteria from the root canal space. Several trials have been done to achieve this goal using different methods, including cleaning and shaping, irrigation techniques and a plethora of intra-canal medications, with varying results.

Although systemic antibiotics appear to be an effective adjunct in most surgical and nonsurgical endodontic procedures, their administration is not without the potential risk of adverse effects, such as allergic reactions, toxicity and the development of resistant strains of microbes. Also, the necrotic root canal is a secluded cavity inaccessible to the local immune system, and the concentration of drug that reaches the canal space after systemic administration of antibiotics is minimal and unlikely to inhibit bacterial growth. Therefore, local application of antibiotics within the root canal system may be a more effective mode for delivering the drug.

Calcium Hydroxide is a highly recommended and widely accepted material for treating intracanal infections. It plays a major role as an inter-visit dressing in the disinfection of the root canal system. Calcium hydroxide cannot be categorized as a

conventional antiseptic, but it kills bacteria in root canal space. [1] The value of calcium hydroxide in endodontic treatment of necrotic infected teeth is now well documented. However, according to literature $Ca(OH)_2$ had a detrimental effect on Dentin micro-hardness [2–4]

Triple Antibiotic Paste (TAP) is a well established and highly efficient antimicrobial agent. It consists of a mixture of metronidazole, ciprofloxacin, and minocycline. But still the development of antibiotic resistant bacterial strains remains a persistent issue, so in an attempt to find a solution to this problem, many have tried naturally found substances as antimicrobials.

Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees.

The medicinal and antimicrobial properties of honey in relation to wound treatment has been recognized for approximately 4500 years, where for instance, King Henry V's head injury was treated with rose honey by John Bradmore, a London surgeon. [5] Honey was the most popular Egyptian drug, being mentioned 500 times in 900 remedies. Prophet Mohammed (P.B.U.H.) and the Roman physician Celsius, used honey as a cure for diarrhea. The

use of honey for treating gastroenteritis was also recorded in ancient China.

The use of honey to treat infections has continued into present-day folk-medicine. In India, lotus honey is traditionally used to treat eye diseases, in Ghana to treat infected leg ulcers, in Nigeria to treat ear-ache, in Mali it is applied on the spots of measles, and in the eyes in measles patients to prevent scarring of the cornea [5]

Therefore, conducting a study to evaluate the potential benefits of using honey as an endodontic intracanal medication was thought of to be of value.

Review of Literature

Peter Molan [6] reviewed the antibacterial effect of honey. They first thought that it was due to its *High sugar content* but due to water dilution and yeast growth it was found to have limited antimicrobial effect. They then assumed that honey's *Acidity* might play a role in the antibacterial properties. Honey naturally has a pH between 3-4.5 due to its gluconic acid content but the concentration of the acid in honey is low, so there is neutralization of the acidity when honey is mixed with wound fluid or saliva and therefore its antibacterial effect is limited.

Further investigations lead to the claim that *Hydrogen peroxide* is the major antimicrobial factor in most honeys.[7] The hydrogen peroxide in honey is produced by the action of the enzyme glucose oxidase which needs Oxygen for the reaction, therefore, the antimicrobial activity from hydrogen peroxide can only be of use where honey is exposed to air. Glucose oxidase is practically inactive in full-strength honey, it becomes active to form hydrogen peroxide only when the honey is diluted [7]. Moreover, investigations have shown that hydrogen peroxide is easily destroyed by heat, light and catalase enzyme, which is produced by honey and bacteria.[5]

There have been many reports of antibacterial activity persisting in honeys treated with catalase to remove hydrogen

peroxide. This is due to the presence of Non- Peroxide components [8] like *Lysozymes* (bacteria destroying enzymes), and *Flavonoids*. Flavonoids are ubiquitous in photo-synthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. They have antifungal, antivral and antibacterial properties. The Antibacterial properties of Flavonoids can be attributed to one or all of these processes, *Inhibition of nucleic acid synthesis, Inhibition of cytoplasmic membrane function* and *Inhibition of energy metabolism.*[9]

Honey is primarily used in wound and burn healing and this is due to its antibacterial, anti-inflammatory, anti-oxidant, osmotic and analgesic properties. It promotes wound healing through *Stimulation of the healing process of chronic wounds, Clearance of infection in wounds, Cleansing action on wounds, Stimulation of Tissue regeneration,* and *Reduction of Inflammation*. [10]**.** The Immuno-activating effect of honey improves the immune reaction of the body. Honey has a proven Anti-fungal activity especially against *Candida albicans*. It also has an Anti-viral (anti-Rubella) properties which makes it a popular component of many cough syrups. Moreover honey applied to skin at the insertion points of medical devices may have a role in the treatment or prevention of infections by coagulase-negative staphylococci making it a promising skin disinfectant.[6] The antibacterial activity of honey is

very much dependant on the floral source, storage and handling of the honey [5].

Manuka honey is produced in New Zealand by bees that pollinate the native manuka bush. Advocates say it treats wound infections and other conditions. [(15)]**.** The major antibacterial component in Manuka honey, in addition to hydrogen peroxide, is a phytochemical component called Methylglyoxal (MGO) [(10)]. MGO is a compound found in most types of honey, but usually only in small quantities. MGO comes from the conversion of another compound – **Dihydroxyacetone** -- that is found in high concentration in the nectar of Manuka flowers. MGO is thought to give manuka honey its antibacterial power. The higher the concentration of MGO, the stronger the antibiotic effect. [(11)]

Jing Lu et al [(12)] explored the effect of New Zealand, Manuka Honey and other honeys on bacterial growth dynamics and cellular morphology. He found that the main antimicrobial factor in Manuka is MGO. But he also concluded that it wasn't the only antimicrobial factor and there are antimicrobial properties in honeys that have very low MGO levels and had the hydrogen peroxide removed by catalase enzyme.

Cokcetin et al [(13)] have developed a scale for rating the potency of Manuka honey. The rating is called UMF, which stands

for Unique Manuka Factor. The UMF rating corresponds with the concentration of MGO. Not all honey labeled as Manuka honey contains significant levels of MGO. To be considered potent enough to be therapeutic, Manuka honey needs a minimum rating of 10 UMF. Honey at or above that level is marketed as "UMF Manuka Honey"

But **Peter Molan** [14] has found that MGO alone is not the only factor responsible for the antimictobial activity of Manuka honey. It is the synergy between MGO and non-antibacterial components of Manuka Honey. This synergy is responsible for half or more of the UMF activity.

Willix et al [15]**,** tested the effectiveness of Manuka honey on The major wound-infecting species of bacteria. Seven major wound-infecting species of bacteria, *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Streptococcus pyogenes,* were studied to compare their sensitivity to the Non-peroxide antibacterial activity of Manuka honey and to a honey in which the antibacterial activity was primarily due to hydrogen peroxide. The Manuka honey had catalase added to remove hydrogen peroxide, so that only the methyl-gyloxal effect (MGO) was being tested. They concluded that the Manuka honey at a

honey concentration of 1.8% (v/v) completely inhibited the growth of *Staphylococcus aureus* over an incubation period of 8h. The growth of all seven species was completely inhibited by both types of honey at concentrations below 11% (v/v).

Almasaudi et al [16] compared the effects of five types of honey against Staphylococcus aureus. The five types of honey (Manuka Honey UMF +20, Manuka Honey UMF +16, Active +10 Manuka Honey, Sidr honey and Nigella sativa honey) were evaluated for their bactericidal/bacteriostatic activities against both methicillin resistant and sensitive S. aureus. The inhibitory effect of honey on bacterial growth was evident at concentrations of 20% and 10% (v/v). Manuka Honey showed the best results. Manuka Honey UMF +20 had a bactericidal effect on both methicillin resistant and sensitive S. aureus. However, Sidr and N. sativa honey exerted only a bacteriostatic effect. The efficacy of different types of honey against S. aureus was dependent on the type of honey and the concentration at which it was administered. Manuka Honey had the best bactericidal activity.

Cooper et al [(17)] tested the antimicrobial effect of Manuka and Pasture honey against *Methicillin-Resistant Staphylococcus Aureus* (MRSA) and *Vancomycin-Resistant Enterococci* (VRE) (super bugs). Strains of MRSA, VSE and *Vancomycin resistant*

Enterococci (VRE) were isolated from infected wounds and from hospital environmental surfaces. Using an agar incorporation technique to determine MIC, their sensitivity to Manuka and Pasture Honey was established and compared to an artificial honey solution. Comparison of MIC values of antibiotic-sensitive strains with their respective antibiotic-resistant strains demonstrated no marked differences in their susceptibilities to honey. They concluded that the inhibition of bacteria by honey is not exclusively due to osmolarity. A possible role for honey in the treatment of wounds colonized by antibiotic-resistant bacteria is indicated.

French et al [18] has made a rather interesting discovery, that pastor honey and Manuka honey successfully inhibited the growth of coagulase negative staphylococci which can usually be found around invasive medical devices. In his study he studied the effect of Pastor Honey and Manuka Honey on clinical isolates of coagulase-negative staphylococci. An agar incorporation technique was used to determine the Minimum Inhibitory Concentration. The growth of all coagulase negative staphylococcus isolates was inhibited by manuka and pasture honeys at concentrations of 2.7–5% (v/v). There was no significant difference between the two types of natural honey for all isolates, including the antibiotic-resistant and antibiotic-susceptible isolates. The results of this study clearly show that manuka honey