Antimicrobial Efficacy of Chitosan Nanoparticles and Ethanolic Propolis Extract against Enterococcus Faecalis and Candida Albicans Biofilm

(In Vitro Study)

Thesis

Submitted to Faculty of Dentistry, Ain Shams University
In Partial Fulfillment of the Requirements for Master's Degree in
Endodontics

By

Dalia Abdel Fattah Abbas Sayed Tantawy

B.D.S. (Future University in Egypt, 2014)

Faculty of Dentistry
Ain Shams University
2019

SUPERVISORS

Prof. Dr. Abeer Hashem Sayed Mahran

Professor of Endodontics

Faculty of Dentistry

Ain Shams University

Prof. Dr. Soha Abdel Rahman ElHady

Professor of Microbiology and Immunology

Faculty of Medicine

Ain Shams University

Acknowledgement

*First and foremost, thanks to **ALLAH** the Most Gracious and the Most Merciful for giving me the strength, knowledge, ability and opportunity to undertake this research study and to complete it satisfactorily. Without his blessings, this achievement would not have been possible.*

*I would like to express my deepest gratitude and appreciation to my thesis supervisor **Prof. Abeer Hashem Sayed Mahran,** Professor of Endodontics, Faculty of Dentistry, Ain Shams University for her extreme heartfelt support, time and effort. Without her passionate help and guidance this study could not have been successfully conducted. It has been a great honour to work under her supervision.*

*I would like to acknowledge **Prof. Soha Abdel Rahman ElHady**, Professor of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her help, time and valuable comments.*

*My special thanks to my second home, the **Endodontic Department at Future University in Egypt**. I must express my deepest gratitude to my friends at Future University in Egypt for their continuous love and encouragement.*

*Last but not least, my acknowledgement wouldn't be complete without thanking the biggest source of my strength, **my family**. The blessings of my beloved parents and the love of my sisters always showed me the way to the right path.*

Dalia Abdel Fattah Tantawy

Dedication

I would like to dedicate this work to my beloved family whom always believed in me. I wouldn't have been where I am today and what I am today without their continuous encouragement and love.

I would like to thank my father, mother and my sisters; Salma, Lamia and Reham with all of my heart and I know they are always sending me their blessings constantly. This would not have been possible without their unwavering and unselfish love and support given to me at all times. I hope I can always make you proud of what I am achieving in my life.

LIST OF CONTENTS

	Page
LIST OF FIGURES	**II**
LIST OF TABLES	**III**
INTRODUCTION	**1**
REVIEW OF LITERATURE	**3**
1.Enterococcus faecalis and its role in root canal infections	**3**
2. Candida albicans and its role in root canal infection	**4**
3. Bacterial biofilm	**5**
4. Endodontic irrigants and natural alternatives	**7**
5. Chitosan	**10**
A. Chemical and biological properties of Chitosan	**10**
B. Antimicrobial activity of Chitosan in endodontics	**12**
6. Propolis	**15**
A. Chemical and biological properties of Propolis	**15**
B. Antimicrobial activity of propolis in endodontics	**19**
AIM OF THE STUDY	**21**
MATERIALS AND METHODS	**22**
RESULTS	**36**
DISCUSSION	**49**
SUMMARY & CONCLUSION	**60**
RECOMMENDATIONS	**63**
REFERENCES	**64**
ARABIC SUMMARY	**-**

LIST OF FIGURES

Fig. No.	Title	Page
1	Diagram showing samples classification	23
2	Showing bacterial inoculation using 1ml insulin syringe	25
3	Samples stored in a closed container before incubation at 37°C	25
4	Zeta potential distribution of Chitosan nanoparticles was 30.7 mV.....	27
5	Transmission electron microscope shows particle size of Chitosan nanoparticles 75.4 nm (red arrow).....	27
6	Filtration was done to get clear solvent	28
7	Showing RevoS system and Traus endomotor	30
8	Zeiss LSM 710 Confocal microscope.....	33
9	CLSM of the positive control group showing green fluorescence indicating live micro organisms.....	34
10	CLSM of the negative group showing red fluorescence indicating dead micro-organisms inside the dentinal tubules.....	34
11	Bar chart showing the antimicrobial effect of different irrigants on the same root level	37
12	Bar chart representing antimicrobial effect of different irrigating solutions on different root levels.....	41
13	Bar chart representing relation between control and experimental groups	43

Fig. No.	Title	Page
14	CLSM showing the effect of Chitosan nanoparticles at the coronal third	**44**
15	CLSM showing the effect of Propolis at coronal third	**44**
16	CLSM showing the effect of NaOCl + EDTA on the coronal third	**45**
17	CLSM showing the effect of Chitosan nanoparticles at the middle third	**45**
18	CLSM showing the effect of Propolis at the middle third	**46**
19	CLSM showing the effect of NaOCl + EDTA on the middle third	**46**
20	CLSM showing the effect of Chitosan nanoparticles on the apical third.....	**47**
21	CLSM showing the effect of Propolis on the apical third.....	**47**
22	CLSM showing the effect of NaOCl + EDTA on the apical third	**48**

LIST OF TABLES

Table No.	Title	Page
1	The mean, standard deviation (SD) of percentage of non-viable microorganisms in experimental groups	**41**
2	The mean, standard deviation (SD) of percentage of non-viable microorganisms in different control and experimental groups	**43**

INTRODUCTION

Endodontic infection is well known to be of a polymicrobial nature full of obligate anaerobic bacteria and fungal species [1].

Therefore, our aim is to provide a root canal system free of any microorganisms to ensure proper healing. Since complete eradication of microorganisms is impossible due to the complexities of the root canal system[2], decreasing the bacterial load using different irrigating solutions could be the solution. Obturation also provides entombing of any remaining resistant bacteria.

Primary endodontic infections are usually caused by many species, dominated by gram-negative anaerobic rods while in secondary infections the microorganisms involved are mainly of one or a few bacterial species [3].

It was concluded that 35% or more of the root canal surfaces remained un-touched regarding the instrumentation technique used [2]. Therefore, irrigation plays an important role in root canal cleaning.

Hence, complete eradication of micro flora from the root canal system ensures the success of the root canal therapy. Several irrigating solutions are being used in endodontic practice [4].

Resistant microorganisms usually remain in root canal complexities even after chemo mechanical preparation of the root canals. This is due to the presence of complex anatomy, accessory canals, and the ability of microorganisms to survive even in harsh conditions [5].