

### THE ANTI-INFLAMMATORY POTENCY OF VITAMIN E AND COCONUT OILS ON LIGATURE-INDUCED PERIODONTITIS IN ALBINO RATS

### Histological and Immunohistochemical Study

#### **Thesis**

Submitted to the Department of Oral Biology, Faculty of Dentistry, Ain Shams University
In partial fulfillment of the requirements for Master Degree in
Oral Biology

### By Hoda Omar Mohamed Helmy Desouky

B.D.S. Faculty of Dentistry, Misr International University, 2011
Demonstrator in the Oral Biology Department
Faculty of Dentistry - Misr International University

### Supervised by

### Prof. Dr. Ahmad Mahmoud Halawa

Head of Oral Biology Department Faculty of Dentistry – Ain Shams University

### Ass. Prof. Dr. Mohamed Ahmed Zayed

Assistant Professor of Oral Biology Faculty of Dentistry – Misr International University

#### Dr. Dina Hazem Hassan Gomaa

Lecturer of Oral Histology
Faculty of Dentistry - Ain Shams University

Faculty of Dentistry Ain Shams University 2019

## <u>Dedication</u>

I would like to dedicate this work to my parents, my siblings, my husband, and my lovely daughter Hana.

# Acknowledgements

All praise is to Allah and all thanks for guiding me and enabling me to fulfill this thesis which I hope will benefit others.

I would like to express my deepest gratitude to **Prof. Dr. Ahmed**Mahmoud Halawa, Professor of Oral Biology, Faculty of Dentistry, Ain

Shams University who gave me his time, effort, experience and continuous guidance throughout this work

I am also thankful for Ass. Prof. Dr. Mohamed Ahmed Zayed,
Assistant Professor of Oral Biology, Faculty of Dentistry, Misr
International University for his untiring support and guidance.

I would also like to thank **Dr. Dina Hazem Hassan Gomaa**,

Lecturer of Oral Biology, Faculty of Dentistry, Ain Shams University for

her encouragement and for giving me her effort, time and experience.

Finally, I am thankful to all members of the Department of Oral Biology, Faculty of Dentistry, Ain Shams University for their support.

## LIST OF CONTENTS

	Page
LIST OF ABBREVIATIONS	III
LIST OF FIGURES	IV
LIST OF TABLES	X
INTRODUCTION & REVIEW OF LITERATURE	1
- The Periodontium	3
- Structural composition of the periodontal ligament	3
- Periodontal Disease	5
- The microscopic nature of periodontal disease	6
- Healing of the periodontal tissues after disease	9
Chlorhexidine	11
- Disadvantages of Chlorhexidine	11
- Herbal Mouthwashes as a Potential Alternative to	13
Chlorhexidine	
- Oil Pulling: Systemic and Oral Benefits	14
- Oil gum massage therapy: a modified oil pulling technique	16
- Vitamin E	17
- The role of antioxidant status in periodontal health	18
- Previous studies showing the antioxidant-related anti-	19
inflammatory effect of vitamin E on periodontitis	
- Coconut oil	20
- Previous studies showing the effect of oil pulling/oil gum	21
massage on periodontal health	

AIM OF THE STUDY	24
MATERIALS AND METHODS	25
RESULTS	36
Histological results	36
Collagen fiber assessment by Masson's Trichrome Staining	71
Immunohistochemical results	95
Statistical analysis	106
DISCUSSION	112
SUMMARY	122
CONCLUSIONS & RECOMMENDATIONS	129
REFERENCES	130
ARABIC SUMMARY	•

## LIST OF ABBREVIATIONS

<b>CD</b> cluster of diff	reginine-naphthylamide Ferentiation lating factor-1 Feleic acid
ANOVA Analysis of v BANA benzoyl-DL- CD cluster of diff	reginine-naphthylamide Ferentiation lating factor-1 Feleic acid
BANA benzoyl-DL-c CD cluster of diff	arginine-naphthylamide Ferentiation Lating factor-1 Feleic acid
<b>CD</b> cluster of diff	Ferentiation lating factor-1 Pleic acid
~~	eleic acid
CSF-1 Colony stimu	eleic acid
<b>DNA</b> deoxyribonuc	
<b>DPX</b> distyrene, a p	lasticizer, and xylene
	soluble proanthocyanidins
<b>ECM</b> extracellular	matrix
<b>EGF</b> Epidermal gr	owth factor
<b>g</b> grams	
<b>H&amp;E</b> hematoxylin	and eosin
I/V Intravenous	
IHC Immunohisto	chemistry
<b>IL-1</b> interleukin-1	
<b>IL-1Ra</b> interleukin-1	receptor antagonist
<b>IL2R</b> interleukin -2	receptor
<b>kg</b> kilograms	
<b>LDL</b> low density la	poprotein
<b>mg</b> milligrams	
<b>ml</b> milliliters	
	oproteinase-1
MMP-9 Matrix metal	
MRC Medical Rese	
MTC Masson's tric	
	breath assessment
<b>PBS</b> Phosphate but	
<b>PDL</b> periodontal li	
	uclear leukocytes
	ator of nuclear factor kappa-B ligand
<b>RBCs</b> red blood cel	
<b>ROS</b> reactive oxyg	
SD Standard dev	
SOD Superoxide I	
	ors of matrix metalloproteinases
	is factor alpha
TRAP Tartrate-Resi	stant Acid Phosphatase

## LIST OF FIGURES

Fig.	Title	Page
1	Schematic representation of the progression of the gingivitis lesion (A) to a periodontitis lesion (B) on the cellular level	7
2	A photograph showing administration of ketamine 10% injection for general anesthesia	28
3	A photograph showing the retraction of the oral cavity using 5-0 silk thread and surgical scissors to allow for the placement of ligatures subgingivally on maxillary first molar	28
4	Photograph showing the upper first molar area of the rat following ligature removal 7 days after their placement	29
5	A photograph showing the maxilla of the rat after it was excised for laboratory processing	29
6	A photomicrograph of a mesiodistal section of the negative control group showing: gingival group, transseptal group, alveolar crest group, horizontal group, oblique group, and apical group. ( <b>H&amp;E x 40</b> )	38
7	A photomicrograph of the periodontal ligament of the negative control group showing the interradicular group of PDL fibers (H&E x100)	39
8	A photomicrograph of the periodontium of the negative control group showing the smooth surface of alveolar bone lined by osteoblasts, osteocytes entrapped in the bone matrix and perforated by Zukerkandl and Hirschfield canal carrying blood vessels into it. (H&E x100)	39
9	A photomicrograph of the periodontium of the positive control group showing the gingival group, transseptal group, alveolar crest group, and horizontal group of PDL fibers ( <b>H&amp;E x100</b> )	42
10	A photomicrograph of the positive control group showing oblique group of PDL. ( <b>H&amp;E x100</b> )	42
11	A photomicrograph of the periodontium of the positive control group showing the interradicular group of PDL. ( <b>H&amp;E x100</b> )	43
12	A photomicrograph of the periodontium of the positive control group showing the apical group of PDL fibers. ( <b>H&amp;E x100</b> )	43
13	A photomicrograph of the periodontium of subgroup A.	46

Fig.	Title	Page
	(H&E x100)	
14	A photomicrograph of chlorhexidine subgroup A showing the interradicular group of PDL. ( <b>H&amp;E x100</b> )	46
15	A photomicrograph of the periodontium of subgroup B showing more dense, regular collagen bundles of the oblique group ( <b>H&amp;E x100</b> )	49
16	A photomicrograph of subgroup B showing alveolar crest group, horizontal group and oblique group. ( <b>H&amp;E x100</b> )	49
17	A photomicrograph of the periodontium of subgroup B showing apical group of PDL fibers. ( <b>H&amp;E x100</b> )	50
18	A photomicrograph of the periodontium of subgroup C showing gingival, transseptal, alveolar crest, horizontal and oblique group of PDL fibers.( <b>H&amp;E x100</b> )	53
19	A photomicrograph of the periodontium of subgroup C showing interradicular fiber group. ( <b>H&amp;E x100</b> )	53
20	A photomicrograph of subgroup C showing apical group of PDL fibers. ( <b>H&amp;E x100</b> )	54
21	A photomicrograph of Vitamin E subgroup A showing transseptal, alveolar crest, horizontal and oblique group of PDL fibers ( <b>H&amp;E x100</b> )	57
22	A photomicrograph of Vitamin E subgroup A showing interradicular PDL fiber group. ( <b>H&amp;E x100</b> )	57
23	A photomicrograph of the periodontium of subgroup A showing apical group of PDL fibers. ( <b>H&amp;E x100</b> )	58
24	A photomicrograph of the periodontium of subgroup B showing oblique fiber group of PDL. ( <b>H&amp;E x100</b> )	60
25	A photomicrograph of the periodontium of subgroup B showing interradicular fiber group of PDL. ( <b>H&amp;E x100</b> )	60
26	A photomicrograph of the periodontium of vitamin E subgroup C featuring oblique PDL fibers. ( <b>H&amp;E x100</b> )	62
27	A photomicrograph of the periodontium of vitamin E subgroup	62

Fig.	Title	Page
	C showing apical fibers of PDL. ( <b>H&amp;E x100</b> )	
28	A photomicrograph of the periodontium of coconut oil subgroup A. ( <b>H&amp;E x100</b> )	65
29	A photomicrograph of the periodontium of coconut oil subgroup A showing interradicular group of PDL fibers. ( <b>H&amp;E x100</b> )	65
30	A photomicrograph of the periodontium of coconut oil subgroup A showing the apical fibers of PDL. ( <b>H&amp;E x100</b> )	66
31	A photomicrograph of coconut oil subgroup B showing the gingival group, transseptal group, alveolar crest, horizontal and oblique group of PDL fibers. ( <b>H&amp;E x100</b> )	68
32	A photomicrograph of coconut oil subgroup B showing the oblique group of PDL fibers. ( <b>H&amp;E x100</b> )	68
33	A photomicrograph of coconut oil subgroup C showing the gingival group, transseptal group, alveolar crest and horizontal group of PDL. ( <b>H&amp;E x100</b> )	70
34	A photomicrograph of coconut oil subgroup C showing the apical group of PDL fibers. ( <b>H&amp;E x100</b> )	70
35	A photomicrograph of the negative control group showing the gingival group, transseptal group, alveolar crest, horizontal and oblique group of PDL. (MTC x100)	72
36	A photomicrograph of the positive control group showing the oblique group of PDL. (MTC x100)	74
37	A photomicrograph of the positive control group showing the apical group of PDL. (MTC x100)	74
38	A photomicrograph of the positive control group showing the interradicular group of PDL. (MTC x100)	75
39	A photomicrograph of the chlorhexidine subgroup A showing the oblique group. (MTC x100)	77
40	A photomicrograph of the chlorhexidine subgroup A showing interradicular group of PDL. (MTC x100)	77

Fig. No.	Title	Page
41	A photomicrograph of the chlorhexidine subgroup A showing the apical fibers of PDL. (MTC x100)	78
42	A photomicrograph of the chlorhexidine subgroup B showing gingival, transseptal, alveolar crest, horizontal, and oblique group of PDL. (MTC x100)	80
43	A photomicrograph of the chlorhexidine subgroup B showing deeply stained, regularly arranged apical fibers. (MTC x100)	80
44	A photomicrograph of the chlorhexidine subgroup C showing gingival group and transseptal group. (MTC x100)	82
45	A photomicrograph of the chlorhexidine subgroup C showing oblique group of PDL. (MTC x100)	82
46	A photomicrograph of the vitamin E subgroup A showing alveolar crest, horizontal and oblique groups. (MTC x100)	84
47	A photomicrograph of vitamin E subgroup A showing the apical fibers of PDL. (MTC x100)	84
48	A photomicrograph of vitamin E subgroup B showing gingival, transseptal, alveolar crest, and horizontal fibers. (MTC x100)	86
49	A photomicrograph of vitamin E subgroup B showing oblique fibers of PDL. (MTC x100)	86
50	A photomicrograph of vitamin E subgroup C showing gingival, transseptal, alveolar crest, and horizontal fibers. (MTC x100)	88
51	A photomicrograph of coconut oil subgroup A showing oblique fibers of PDL. (MTC x100)	90
52	A photomicrograph of coconut oil subgroup A showing interradicular fibers of PDL. (MTC x100)	90
53	A photomicrograph of coconut oil subgroup B showing oblique fibers of PDL. (MTC x100)	92
54	A photomicrograph of coconut oil subgroup B showing interradicular fibers of PDL. (MTC x100)	92
55	A photomicrograph of coconut oil subgroup C showing	94

Fig.	Title	Page
	gingival and transseptal groups of PDL. (MTC x100)	
56	A photomicrograph of a mesiodistal section of the gingiva of a rat from the negative control group. (anti-MMP-1 x400) counterstain: Hematoxylin.	96
57	A photomicrograph of a mesiodistal section of the gingiva of a rat from the positive control group. (anti-MMP-1 x400) counterstain: Hematoxylin	96
58	A photomicrograph of a mesiodistal section of the gingiva of chlorhexidine subgroup A. (anti-MMP-1 x400) counterstain: Hematoxylin	98
59	A photomicrograph of a mesiodistal section of the gingiva of chlorhexidine subgroup B. (anti-MMP-1 x400) counterstain: Hematoxylin	98
60	A photomicrograph of a mesiodistal section of the gingiva of chlorhexidine subgroup C. (anti-MMP-1 x400) counterstain: Hematoxylin	99
61	A photomicrograph of a mesiodistal section of the gingiva of vitamin E subgroup A. (anti-MMP-1 x400) counterstain: Hematoxylin	101
62	A photomicrograph of a mesiodistal section of the gingiva of vitamin E subgroup B. (anti-MMP-1 x400) counterstain: Hematoxylin	101
63	A photomicrograph of a mesiodistal section of the gingiva of vitamin E subgroup C. (anti-MMP-1 x400) counterstain: Hematoxylin	102
64	A photomicrograph of a mesiodistal section of the gingiva of coconut oil subgroup A. (anti-MMP-1 x400) counterstain: Hematoxylin	104
65	A photomicrograph of a mesiodistal section of the gingiva of coconut oil subgroup B. (anti-MMP-1 x400) counterstain: Hematoxylin	104

Fig.	Title	Page
66	A photomicrograph of a mesiodistal section of the gingiva of coconut oil subgroup C. (anti-MMP-1 x400) counterstain: Hematoxylin	105
67	Column chart illustrating mean area fraction in different groups	108
68	Column chart illustrating mean area fraction in observation times	111

## LIST OF TABLES

Table No.	Title	Page
1	Comparison of area fraction (%) of immunoexpression between different groups at each observation time (ANOVA test)	107
2	Comparison of area fraction (%) of immunoexpression within the same groups in different observation time (ANOVA test)	110

### **ABSTRACT**

Overview: Plaque induced periodontitis is the second most common oral disease worldwide after dental caries. Adjunctive methods such as mouthwashes have been developed as an aid to routine scaling for treating periodontal inflammation. Aim: The aim of this study was to evaluate the anti-inflammatory potency of vitamin E and coconut oils when used for oil gum massage therapy in periodontitis and compare them to conventionally used chlorhexidine. Methods: 105 adult male albino rats were divided into 5 groups of 21 rats each: The negative control group (group 1) that received no intervention, the positive control group (group 2) that received ligature induced periodontitis without any treatment, the chlorhexidine experimental group (group 3) that received ligature induced periodontitis and chlorhexidine for 10 days, the vitamin E experimental group (group 4) that received ligature induced periodontitis and vitamin E for 10 days. And the coconut oil group (group 5) that received ligature induced periodontitis and coconut oil for 10 days. Groups 2-5 were further divided into subgroups A,B, and C according to day of termination (day 3, 7, &10). After rats were terminated, maxillary molar regions were excised and stained with H&E and Masson's trichrome stain. Specimens were also examined immunohistochemically for the expression of MMP-1 antigen. **Results:** Histological and immunohistochemical examination showed an inflammatory reaction with ligature induced periodontitis that decreased gradually from day 3 until day 10. Statistical analysis regarding the immunohistochemical expression of MMP-1 inflammatory marker showed that it gradually decreased over the course of the treatment in all experimental groups with chlorhexidine showing the greatest decrease in inflammation while there was no significant difference between vitamin E and coconut oil. Conclusion: Vitamin E and coconut oil are effective in reducing periodontal inflammation in rats but less effective than chlorhexidine.

### INTRODUCTION

Plaque induced periodontitis is the second most common oral disease worldwide after dental caries (**Petersen 2003**). Mechanical removal of plaque via routine scaling is critical to maintain the health of the periodontal tissues (**Sharma et al., 2004**).

However, since most people do not consistently control plaque accumulation adequately, adjunctive methods such as antimicrobial mouthwashes and dentifrices have been developed for treating periodontal inflammation (Nadkerny et al., 2015).

Among these adjunctive methods, chlorhexidine has long been considered the "gold standard" for treating gingival inflammation, owing to its broad spectrum action on plaque-causing bacteria (Indurkar et al., 2016).

Since it has been found that most tissue destruction in periodontal disease is dependent more on the host's response to the plaque-causing microorganism rather than the microorganism itself (Lamster & Novak, 1992; Dahiya et al., 2013), the use of antioxidant rich oils for oil pulling and oil gum massage can be seen as a potential alternative to chlorhexidine. Vitamin E oil and coconut oil are two natural products that are currently gaining popularity for their powerful antioxidant properties, enabling them to oxidize free radicals and reactive oxygen species generated during periodontal inflammation (Atalay et al., 2000; Singla et al., 2014).