UNIVERSITI TEKNOLOGI MARA

EFFECTS OF ELLAGIC ACID ON EXTRACTED TOOTH SOCKET HEALING IN NICOTINIC DIABETIC RATS

MAZEN.M.JAMIL AL-OBAIDI

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Dentistry

August 2015

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledgment as referenced work. This thesis has not been submitted to any other academic or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of student	:	Mazen. M. Jamil Al-Obaidi
Student I.D. No	:	2011403912
Programme	:	Doctor of Philosophy
Faculty	:	Faculty of Dentistry (DS990)
Thesis Title	:	Effects of Ellagic Acid on Extracted Tooth Socket Healing in Nicotinic Diabetic Rats
Signature of Student	:	
Date	:	August 2015

ABSTRACT

The resorption of alveolar bone that occurs after tooth extraction leads to many esthetic and functional problems. Ellagic acid (EA) is a member of the flavonoid family that regulates various processes of bone function. Statins are speculated to increase bone formation. This study was designed to evaluate the healing of extracted tooth sockets in diabetic and non-diabetic rats administered with EA orally and treated with rousavastatin (RSV) locally in nicotinic and non-nicotinic rats. Sixty-four male *Sprague–Dawley* rats weighing 250-300 g were selected to conduct the tooth extraction experimental study. The rats were divided into two main groups. The first main group was considered as the non-diabetic group and divided into four sub-groups; (A) The tooth rat socket filled with RSV+ The rats treated with NaCl orally (RSV+NaCl). (B) The tooth rat socket filled with RSV + the rats treated with EA orally (RSV+EA). (C) The tooth rat socket filled with RSV + The rats injected with Ni (RSV+Ni). (D) The tooth rat socket filled with RSV + the rats injected with Ni + treated with EA (RSV+Ni+EA). The second main group was considered as the diabetic group and divided into four sub-groups: (A) The tooth rat socket filled with RSV+ the rats treated with NaCl orally (RSV+NaCl). (B) The tooth rat socket filled with RSV + the rats treated with EA orally (RSV+EA). (C) The tooth rat socket filled with RSV + The rats injected with Ni (RSV+Ni). (D) The tooth rat socket filled with RSV + the rats injected with Ni + treated with EA (RSV+Ni+EA). Both main groups were intraperitoneally anesthetized with 0.08 ml of xylazine and 0.17 ml of ketamine, and the upper left central incisor was then extracted. Subsequently, the whole sockets were filled with 10 mg/kg RSV and closed with a dental resorbable suture. Immunohistochemical technique (IHC) was performed on the serial sections to assess the healing process using several biomarkers. These biomarkers include anti-transforming growth factor beta 1, anti-vascular endothelial growth factor (VEGF), anti-proliferating cell nuclear antigen (PCNA), anti-fibroblast growth factor 2, anti-alkaline phosphatase (ALP), anti-osteocalcin, and anti-bone sialoprotein antibodies (BSP). Serum tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) were measured using the ELISA kit before and after the experiment. Malondialdehyde (MDA) levels as well as superoxide dismutase (SOD) and catalase (CAT) activities were determined in the homogenized gingival tissue of rats at the end of the experiment using a commercial kit. Results showed that the expression levels of TGF-β, VEGF, PCNA, FGF-2, ALP, OCN and BSP significantly increased following the EA administration in diabetic (RSV+EA) and diabetic nicotinic rats (RSV+Ni+EA) compared with those of untreated diabetic (RSV+NaCl) and diabetic nicotinic rats (RSV+Ni) at day 14 ($P<0.05$). A decrease in MDA levels and a significant increase in the CAT and SOD activities of nicotinic and diabetic rats were observed following the EA treatment when compared with untreated nicotinic and diabetic rats ($P<0.05$). Pro-inflammatory factors TNF-α and IL-6 significantly elevated in nicotinic and diabetic rats and then decreased after EA treatment ($P<0.05$). We conclude that oral administration of EA adjunct with RSV exerted a positive effect on bone formation and bone remodeling biomarkers. EA with RSV may provide a promising line of treatment for nicotinic and diabetic patients after tooth extraction.

ACKNOWLEDGEMENTS

I have barely passed through a small, but one of the toughest gates in my life. I would not have accomplished this work without God's blessing and so many kinds of help from lovely people.

The first and the most, I would like to express sincere gratitude to my supervisors Prof Dr. Fouad Hussain Al-Bayaty and Prof Dr Mahmood Ameen Abdulla for their guidance, patience and support for me to make this goal come true. I would like to express special thanks to Dr. Jamal Hussaini, for his support for my research at Universiti Teknologi Mara, Faculty of Medicine in animal house. My gratitude also extends to Dr. Omar for his assistance and advice while working on my research projects in histology part. I would also like to thank Dr. Aqil M Daher for his support in statistics part. I would like to acknowledge the members of the staff and fellow graduate students at UM & UiTM.

I would like to extend my appreciation and respect to my parents for their assistance and incredible moral and financial support over the course of this study. I am grateful to my mother for her belief in my ability and her support. I love you and thank you. Last, I would like to express thanks to my father, Muhanned Jamil and his biggest legacy, passion and belief in the life.

I am grateful to my wife (Farah Aamer) and daughters (Yara & Leyan) for their sacrifice, patience and understanding that we are inevitable to make this work possible. I cannot find the appropriate words that could properly describe my appreciation for their devotion, support and faith in my ability to attain my goals.

Finally, yet importantly, I acknowledge the financial support for this study by Universiti Teknologi MARA (600-RMI/DANA 5/3/RIF (574/2012).

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The resorption of alveolar bone that occurs following tooth extraction is known as Residual ridge resorption (RRR). Residual ridge resorption is chronic, progressive, irreversible, and accumulative. It leads to many aesthetic and functional problems in edentulous patients because of the continuous resorption in the alveolar bone. Sufficient alveolar bone volume and favorable architecture of the alveolar ridge are essential to obtain ideal, functional, and aesthetic prosthetic reconstruction after implant therapy (Schropp et al., 2003).

Diabetes mellitus (DM) is a common disease that causes significant mortality and morbidity. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia due to the inability to produce insulin (Zimmet et al., 2003). The World Health Organization has estimated that in 2030, Malaysia would have a total number of 2.48 million diabetics compared with 0.94 million in 2000, thereby showing a 164% increase (Mafauzy, 2006). Hyperglycemia hampers bone formation, thereby leading to a poor outcome for diabetic individuals (Mellado Valero et al., 2007). Moreover, the healing of the tooth extraction socket in poorly controlled diabetic patients is often delayed and accompanied by infection (Bell et al., 1999). Clinical and animal studies have shown a positive correlation between DM and periodontal disease (He et al., 2004), thereby impairing extra-oral/skin wound healing (Peppa et al., 2003) and the development of perpendicular lesions (Iwama et al., 2003).

Nicotine (Ni) is one of more than 4700 compounds found in unfiltered mainstream tobacco smoke (Hoffmann et al., 2001). The adverse effects of smoking on bone healing have been widely reported. Cigarette smoke contains high concentrations of oxidants and free radicals (Pryor & Stone, 1993) and is well known as one of the exogenous factors of