FABRICATION OF DOXYCYCLINE-*NIGELLA SATIVA*-EUGENOL (DNE) EMULSION INTENDED FOR LOCAL TREATMENT OF CHRONIC PERIODONTITIS

BY

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ABSTRACT

Locally applied antibiotic in periodontal therapy offers a low-cost, non-invasive procedure with potential to maximise therapeutic efficacy with a low drug dosage regimen. In this study, newly fabricated doxycycline hyclate (DH) emulsions which differ in the amount and type of emulsifiers (lecithin and hydroxypropyl methylcellulose) were thoroughly assessed for their compatibility, characterisation and analytical method. The compatibility assessment employed differential scanning calorimetry (DSC) and attenuated total reflectance – Fourier transform infrared spectroscopy (ATR – FTIR). The emulsions were subjected to phase separation and accelerated stability studies and characterised for droplet size, polydispersity index (PDI) and zeta potential using a nanosizer. Additionally, viscosity and rheological behaviour were also evaluated using a rheometer. An analytical method using HPLC was then developed and validated to quantify doxycycline from the emulsion. A UV-spectrophotometry was also developed and validated and used to quantify release behaviour. It was found that all excipients tested were compatible with each other. Compatibilities of DH with all excipients incorporated in the emulsion were indicated by the consistent thermogram of the DH as shown by DSC for binary mixtures (DH-excipients) of which the excipients tested were sodium propyl paraben, sorbitan monooleate, lecithin, *Nigella sativa* oil, hydroxypropyl methylcellulose, polysorbate 80, sodium methyl paraben and eugenol. The emulsions were also highly stable as there was absence of phase separation after being challenged with centrifugation at 4000 rpm for 15 minutes. Stability was indicated further by the zeta potential values of between -48.2±0.4 to -64.0±3.9. In addition, droplet size was within the range of 198.6±8.2 to 279.3±10.7 nm. The emulsions were also highly polydisperse as PDI value falls between 0.448±0.026 to 0.710±0.080. An increasing and improving pattern of viscosity and rheological behaviour was observed as the concentration of the emulsifiers was increased. The recovery of the doxycycline was found to be 98.2±2.2 % indicating that the purity of the API was maintained following combination with excipients. The in vitro release pattern was conducted using dissolution tester and analysed by UV-spectrophotometry module. The release profile showed a prolonged release (5 hours) in the lecithin stabilized emulsion as compared to lecithin-HPMC stabilized emulsion (2 hours). In conclusion, the stable fabricated emulsion was expected to provide promising therapeutic efficacy as a new locally applied antibiotic for the treatment of periodontitis.

Keyword: Doxycycline hyclate, periodontitis, local drug delivery, emulsion

خلاصة البحث

إن مضادات الالتهاب المستخدمة موضعيا في علاج امراض اللثة قد تكون خيارا رخيص السعر، وطريقة العلاج لا تحتاج الى ادوات للاستخدام مع فعالية دوائية مضاعفة، كما يمكن استخدامه لمرات اقل. في هذا المشروع تم بحث شكل دوائي جديد حيث تم تصنيعه باستخدام دواء الدوكسيسايكلين (دي أتش) لانتاج مستحلب الدوكسيسايكلين الذي يختلف في الكمية ونوع المواد المستحلبة (ليسيثين وهيدروكسي بروبايل ميثايل سليلوز) الذي تم اختباره بعناية في فحص التوافقية في الوصف والطرق التحليلية. تم دراسة فحص التوافقية باستخدام المسح الحراري التفاضلي وفحص طيف الاشعة تحت الحمراء. تم خضوع مستحلب الدوكسيسايكلين الى فحص الوقت اللازم لفصل الماء عن الزيت، وفحص الاستقرارية المسرع وكما تم فحص حجم قطرات مستحلب الدوكسيسايكلين، فحص مؤشر التشتت المتعدد، وفحص الجهد الكهربائي للمستحلب باستخدام جهاز النانو. تم اختبار اللزوجة والجريان للمستحلب تم باستخدام جهاز الريوميتر. تم تطوير وتاكيد طريقة تحليل اخرى للمستحلب باستخدام جهاز الضغط العالي لفصل السوائل الملونة لتحديد كمية الدوكسيسايكلين في المستحلب كما انه تم تطوير وتأكيد طريقة فحص طيف الاشعة تحت الحمراء للتاكد من تحرر كمية الدوكسيسايكلين من المستحلب. نتائج البحث اظهرت ان جميع المواد المضافة والمستخدمة في مستحلب الدوكسيسايكلين كانت متوافقة مع بعضها. نتائج توافقية دواء الدوكسيسايكلين باستخدام المسح الحراري التفاضلي مع المواد المضافة مثل (صوديوم بروبايل بارابين, سوربيتان مونوليت، ليسيثين، زيت الحبة السوداء، هيدروكسي بروبايل ميثايل سليلوز، بولي سوربيت 80 ، صوديوم ميثايل بارابين، ويوجينو. مستحلب الدوكسيسايكلين كان مستقرا كما انه لا يوجد اي علامة لفصل الماء عن الزيت بعد ان وضع المستحلب في جهاز الطرد المركزي لمدة 15 دقيقة وبسرعة 4000 دورة في الدقيقة. نتيجة فحص الجهد الكهربائي للمستحلب كانت من -48.2 ± 0.2 الى -64.0 ± 3.9. اضافة الى ذلك، فإن حجم قطرات المستحلب كان بين 198.6 ± 8.2 الى 279.3 ± 10.7 نانومتر. نتيجة فحص مؤشر التشتت المتعدد كانت 0.448 ± 0.026 الى 0.710 ± 0.080. بازدياد تركيز المواد المستحلبة المضافة، نتيجة اللزوجة والجريان للمستحلب زادت ايضا. نقاهة دواء الدوكسيسايكلين كانت 98.2 ± 2.2 %، حيث دلت هذه النتيجة على نقاوة عالية. فحص تحرير دواء الدوكسيسايكلين من المستحلب كان قد تم باستخدام جهاز التحلل وتم تحليل النتائج باستخدام جهاز طيف الاشعة تحت الحمراء حيث اظهرت النتائج ان دواء الدوكسيسايكلين تم تحريره خلال فترة طويلة (5 ساعات) باستخدام الليسيثين كمادة مستحلبة مقارنة باستخدام الليسيثين مخلوط مع هايدروكسي بروبايل ميثايل سليلوز (ساعتان). اخيرا تم الاستنتاج بان مستحلب الدوكسيسايكلين يمكن ان يكون علاج موضعي واعد وفعال لمحاربة البكتيريا في علاج امراض اللثة.

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APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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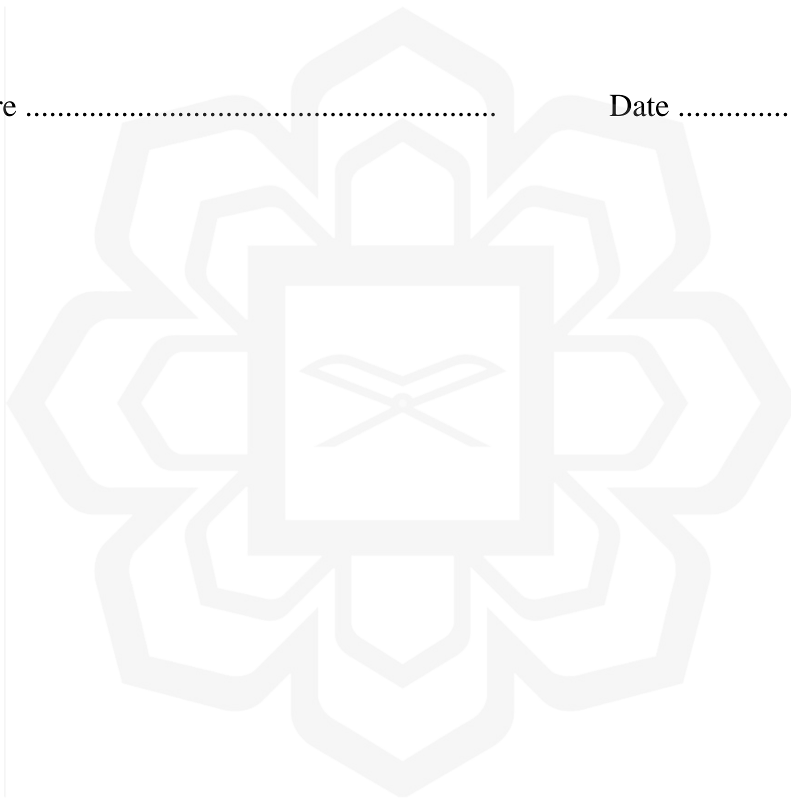

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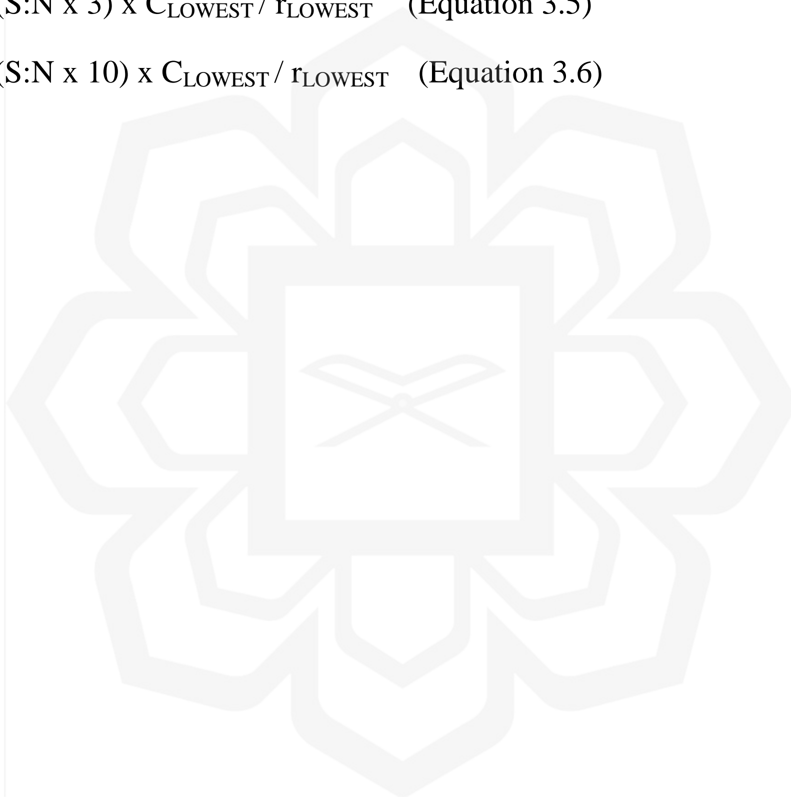

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Chronic periodontitis (CP) is an inflammatory disease due to oral infection affecting teeth-supporting tissues such as the gingiva, periodontal ligament and the alveolar bone and can cause the loss of alveolar bone as well as the destruction of the connective tissues which finally leads to tooth loss (Isola et al., 2018). The periodontal tissue destruction is mainly brought about by the breakdown of periodontal ligament, chiefly by the action of matrix metalloproteinases (MMPs) (Preshaw et al., 2004). The inflammation originates from the microbial biofilm infection on the tooth surface. Due to the frequent exposure to food, change in lifestyle to high sugar diet, limited access of the tooth area for effective treatment and the virulence of the pathogenic bacteria colony, it is challenging to prevent and treat the development of oral biofilm infection and hence, the progression of periodontitis.

Data shows that periodontal disease which includes severe periodontitis is the 6th most common disease worldwide. It affects at least 743 million people with an overall prevalence of 11.2%. It is the major cause of tooth loss in the population of adult (Tonetti, Jepsen, Jin, & Otomo-Corgel, 2017). Global Burden of Disease Study (GBD, 1990-2010) reported an increase of periodontal disease as much as 57.3% within 1990 and 2010 (Tonetti et al., 2017).

In Malaysia, the Ministry of Health conducts an oral health survey for every 10 years. The survey found that 48.5% of Malaysian population is suffering from moderate and severe periodontitis (Dom, Ayob, Muttalib, & Aljunid, 2016). Surveys done by Dom et al. (2013) revealed that the prevalence showed a decline between

1990 and 2000 but rises again from 87.2% to 94% in a survey conducted in 2010. They also found an increase of 13% in patients who needed complex periodontal care from 1990 to 2010.

CP, therefore requires novel approaches to an effective treatment for the patient and minimize the current prevalence (Dom et al., 2016). Previously, there were two major categories for conventional therapeutic approaches. Firstly, the anti-infective treatment which halt the disease progress by removing the aetiological factors and secondly, the regenerative therapy that restores the destroyed structures (Sapra, Patel, Patel, & Borkhataria, 2014). A new local delivery system of the antimicrobial as the adjunctive therapy in various types of dosage forms i.e. fibers, films, injectable systems, gels and strips has been reported to give a more constant and prolonged therapeutic effects to the patients (Sapra et al., 2014).

The application of antimicrobial agents is more favourable in managing the accumulation of biofilm layers of the oral cavity in comparison to the mechanical plaque control procedures (Lakhdar et al., 2012), therefore in this study, a doxycycline-based emulsion is formulated and tested in the hope it can reduce, if not totally do away with mechanical, surgical procedure against biofilm periodontitis. The doxycycline emulsion is a fusion formulation combining doxycycline with *Nigella sativa* and eugenol. This combination of natural products with conventional antibiotic is believed to have a synergistic effect allowing delivery through local drug delivery system with prescription at a lower dosage which reduces the toxicity effect.

By employing a local drug delivery system the drug can be directly inserted into the base of the intraperiodontal pocket which is often inaccessible to contemporary dental instruments. Inadvertently, this reduces the required drug dose. The formulation

is then expected to reside in the pocket for the antimicrobial effect to take place (Kopytynska-kasperczyk, Dobrzynski, Pastusiak, Jarzabek, & Prochwicz, 2015).

The formulation is studied thoroughly in order to ensure its quality and to meet regulatory requirements for drug-like properties. As such the study began at the earliest stage of the formulation's fabrication, i.e. the pre-formulation stage, in which the drug-excipient compatibility assessment will be carried out.

In addition to the compatibility assessment, the fabricated formulation has to be subjected for investigations in order to know the nature and properties of the formulations (Maiti et al., 2016). Therefore, systematic approaches; qualitative approach and quantitative approach were adopted for the evaluation processes. These includes characterization for all parameters like the particle size distribution, zeta potential and viscosity as well as the quantification of the doxycycline in the emulsion and its release behaviour.

1.2 Literature Review

1.2.1 Chronic Periodontitis (CP)

Healthy teeth are maintained in the maxillary and mandibular bones by specialized tissues in the oral cavity. These teeth-supporting tissues are alveolar bone, gingival (gums), the root cementum and the periodontal ligaments (Dom et al., 2013). The impairment of the teeth-supporting structure by the pathogenic bacteria can cause symptoms like gum pain, swelling, bleeding, abscesses, mobility of teeth within socket and finally lost of tooth (Mohd-dom et al., 2013). This constitutes what is called chronic periodontal disease.

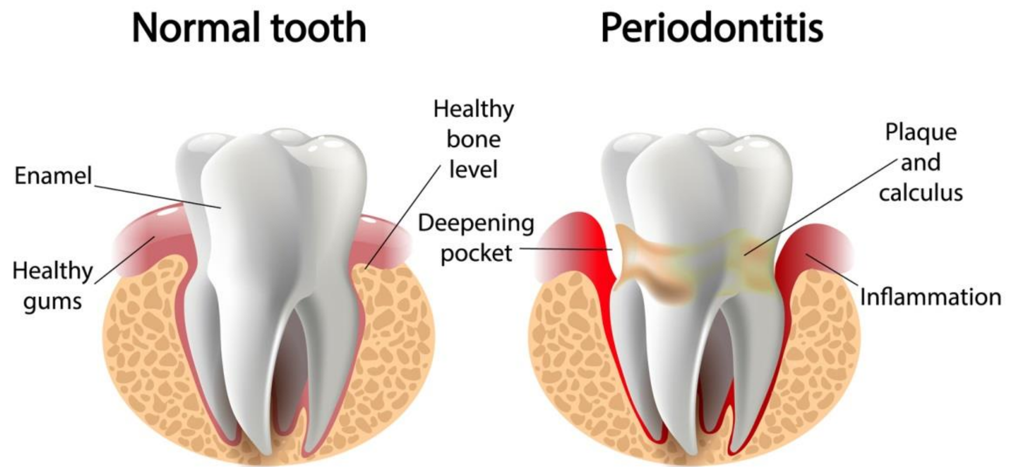

Figure 1.1 Comparison between a normal tooth and periodontitis showing inflammation, deepening of gingival pocket (sulcus) and presence of plaque and calculus. Adapted from Periodontitis: why we need a vaccine for gum disease, Darby I., Retrieved August 20, 2017, from http://theconversation.com/periodontitis-why-we-need-a-vaccine-for-gum-disease-69978.

Chronic periodontitis is the more advanced inflammatory form of periodontal disease, in which breakdown of the supporting tissues of the teeth occurs by local factors such as dental biofilm (Petersen & Ogawa, 2012). According to a review by Hajishengallis (2013), the virulent bacteria that adhere to the tooth surfaces are commonly *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* or better known as the 'red complex'. In aggressive periodontitis, these are highly associated with *Aggregatibacter actinomycetemcomitans* (Jiao, Hasegawa, & Inohara, 2014).

The clinical signs of CP include deepening of periodontal pockets and loss of teeth attachment, progressively leading to loosening of teeth and ultimately to tooth loss (Petersen, 2012). The periodontal pocket is the gap between the gingiva and tooth

and this gap provides an ideal condition for the proliferation of microbes. The bone resorption would be halted and the depth of the diseased pockets can be reduced when the amount of the pathogenic bacteria is reduced through periodontal therapy (Sapra et al., 2014). Thus, the application of antimicrobial agents is of priority in managing the accumulation of biofilm layers of the oral cavity (Lakhdar et al., 2012).

1.2.2 Periodontal Therapy and Its Cost

As periodontal disease currently is the world's sixth most common disease, oral disease treatment unsurprisingly is the fourth most expensive disease in many industrialized countries. The cost is often shouldered by the government (Dom et al., 2014; Dom et al., 2016; Umeizudike, Iwuala, Ozoh, Ayanbadejo, & Fasanmade, 2016; Tonetti et al., 2017). Dom et al. (2014) in his study reported that on each Malaysian patient would cost as much as MYR 2820 annually and MYR376 per outpatient visit. From this figure, 90% is subsidized by the Malaysian government: a whooping MYR 29.1 billion alone was set aside for the year 2012 for the management of periodontitis among its citizens. An incline in the prevalence of periodontitis has caused an economic burden as the cost has exceeded from the budget allocated to the MOH by as much as 60.6% (Dom et al., 2016). They also reported that the cost for the treatment rises as the severity of the disease worsens.

The treatment offered to the patient varies according to the severity of the disease. There are 4 phases of periodontal therapy which includes non-surgical, surgical, restorative and maintenance. In summary, Sapra et al. (2014), wrote that there are two categories of the conventional periodontal therapy; anti-infective treatment (surgical) and regenerative therapy (restorative). The anti-infective treatment is created to retard

the progression of periodontal attachment loss through the removal of the aetiologic factors while the regenerative therapy is aimed for the restoration of the destroyed structure.

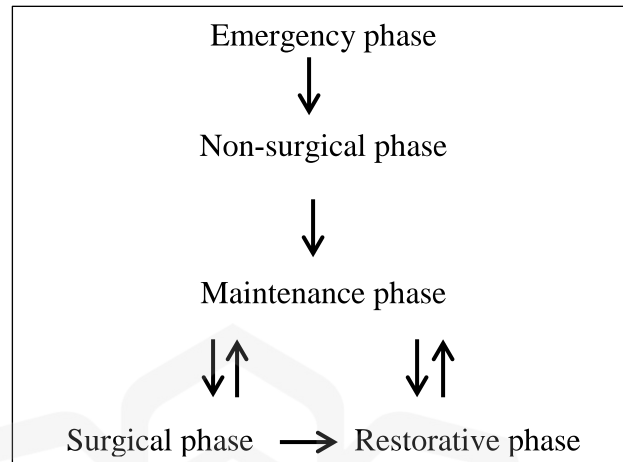

Figure 1.2 The sequence of periodontal therapy (Newman, Takei, Klokkevold, Carranza, 2007)

The mechanical debridement of the root surface is invasive and costly. This comes together with other limitations like the inability to access the deep pockets, and the possible recolonization of pathogens. This is so because the mechanical debridement alone often leaves behind significant number of pathogens due to possible instrumentation limitations or the ability of microorganism to penetrate into deeper tissues (Herrera, Matesanz, Martinez & Sanz, 2012; Aviral et al., 2012). Scaling root planning also has attendant risks of severe bleeding and discomfort feeling.

Locally applied delivery system (nonsurgical) which contains antimicrobial has also been introduced. In this type of delivery system, antibiotics will be placed directly at the site of infection, thus, producing prolonged therapeutic duration towards the pathogenic bacteria. There are various kinds of dosage forms formulated such as strip, films, fibers, gels, vesicular system, nanoparticles and microparticles.