

Review: exploring the potential of *Nigella sativa* for tooth mineralization and periodontitis treatment and its additive effect with doxycycline

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Abstract

Oral disease has been a worldwide concern as the incidence and cost of treatment continues to rise. Management of the disease is challenging as success is often influenced by an individual's lifestyle and diet. *Nigella sativa* (*N. sativa*) or black seed is a medicinal plant that has received growing interest due to its effectiveness against various conditions including cardiovascular disease, diabetes, cancer, as well as infectious diseases caused by bacterial, viral, and fungal infections. This narrative review studies *N. sativa* that has also shown great potential in dental health attributed to its role in stimulating the process of remineralization. Apart from that, *N. sativa* promotes healing of oral tissues as it induces the differentiation potential of heterogeneous cell populations in periodontal ligament cells. The anti-osteoporotic, antioxidant and anti-inflammatory properties of *N. sativa* also improve periodontal healing, particularly by preventing further destruction of bone components. This article discusses the occurrence of periodontitis and the therapeutic role of *N. sativa* with emphasis on the regulation of genes, for instance, the alkaline phosphatase (*ALP*) that is involved in tissue specialization and remineralization. It is speculated that the activity of *N. sativa* in remineralization can be enhanced by combination with other commonly used periodontitis antibiotics such as doxycycline. *N. sativa* is purported to induce periodontal tissue regeneration whilst minimizing toxicity. Thus, it may serve as an alternative or enhance the existing treatment for periodontitis when administered as a combined formulation.

Keywords: doxycycline, gene expression, mineralization, *Nigella sativa*, periodontitis

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Introduction

Periodontal disease and dental caries affect up to 50% of the worldwide population. The

percentage of affected individuals are higher among adults and older group of people (Nazir, 2017). The economic impact, whether directly or indirectly, due to

periodontal disease treatments, accounted for about 442 billion USD per year (Tonetti *et al.*, 2017). These data portray the serious implication of periodontal disease not only to an individual's oral health but also financial status. The Malaysian healthcare system, through its dental services, spent approximately RM 2,800 per patient to treat periodontal diseases (Mohd-Dom *et al.*, 2013). A study by Jin *et al.* (2016) stated that chronic periodontitis is among the global disease burden and affected almost 12% of people worldwide between the years 1990 to 2010.

Periodontal disease or gum disease can be characterized into two conditions which include gingivitis and periodontitis. Gingivitis is the mildest form that causes the gum to be red, swollen and tend to bleed easily. This condition is often reversible as the main cause of gingivitis is poor oral hygiene (Shaw *et al.*, 2016). Nevertheless, if gingivitis is left untreated, eventually periodontitis may develop. This occurs due to plaque accumulation reaching beyond the gum line. The presence of bacteria and its toxin initiates an inflammatory response, and eventually, chronic inflammation in the gingiva will slowly destroy the gum tissue and teeth, gingival, periodontal ligament, cementum, and the alveolar bone. As the gingival tissue is inflamed, the tissue itself loses its attachment to the tooth surface, creating a periodontal pocket (Arigbede *et al.*, 2012).

Periodontal disease can be classified into four categories. Firstly, aggressive periodontitis where there is a rapid attachment loss between gingiva and tooth as well as alveolar bone destruction. Secondly, chronic periodontitis which involves inflammation of the periodontium, and this causes the progressive loss of attachment and bone destruction. The main difference between aggressive and chronic periodontitis is the onset. Aggressive periodontitis tends to occur earlier as compared to chronic periodontitis. Wiebe and Putnins (2000) mentioned that the three criteria that govern the categorization are the depth of the pocket, the clinical attachment loss and the number of teeth

affected whether more or less than 30%. The third type of periodontitis may be the result of a systemic disease including respiratory disease, heart disease and diabetes. The effects are suggested to occur due to the infection of bacteria in systemic circulation and cytokine storm released from the extensive inflammatory response. The fourth type is necrotizing periodontitis which affects mostly immunocompromised and malnourished individuals. This condition is attributed to necrosis of the periodontium, gingival tissue, periodontal ligament, and alveolar bone (Wiebe & Putnins, 2000).

Currently, gingivitis can be prevented and treated by advocacy of proper oral hygiene such as frequent tooth brushing. However, for periodontitis, a comprehensive periodontal treatment is required, which may include a non-surgical procedure called scaling and root planing (SRP). This procedure is performed by a dentist to scale all the plaque, bacterial toxins and tartar deposition on the tooth and root surfaces followed by root planing. Root planing smoothens out the surfaces and prevents any re-adherence of plaque or tartar, allowing a condition that induces the healing processes and reattachment of the gum to the tooth (Smiley *et al.*, 2015). SRP is also referred to as deep cleaning, a gold standard in treating periodontitis in which the procedure is commonly coupled with treatment using tetracycline antibiotics, specifically the doxycycline hyclate (Tomasi & Wennström, 2004). Although the treatment is a common practice, the procedure has been reported to be technically challenging and may cause recurrence. The problem with current treatment is that SRP itself induces pain to the patients and may cause patients to retract from getting treatment. The second problem is the presence of bacterial biofilm at the site of treatment. Bacterial biofilm which is embedded in layers has optimal defence against antibiotic effect when compared to the planktonic bacteria (Dincer *et al.*, 2020). Due to the resistant properties, higher doses of antimicrobial as high as 10 to 1000 times will be required than normal to kill it (Gebreyohannes *et al.*, 2019). However,

the administration of drugs at high dosage can cause local toxicity affecting the surrounding area (Shaddox & Walker, 2010). This, in turn, hinders the remineralization of tooth and dentin structures (Holmes & Charles, 2009; Ioannou *et al.*, 2010).

Continuous studies were conducted to improve the treatment for periodontitis (Alshareef *et al.*, 2020; Shaddox & Walker, 2010). It is postulated that an effective therapeutic strategy is to provide a comprehensive treatment that could deliver antimicrobial protection, reduce inflammation, and stimulate remineralization of the alveolar bone. The use of phytomedicine has gained a lot of interest particularly in targeting a wide range disease with more accessible, cost-effective, and fewer side effects in comparison to the synthetic medicines (Yimer *et al.*, 2019). Among medicinal plants, *N. sativa* has been considered one of the highly valued nutrient-rich plants in history around the world. A lot of studies are progressively conducted to validate its traditionally claimed benefits and use (Ramadan, 2007).

Research on the use of traditional medicines opens new boundaries for the alternative approach in the management of oral diseases (Cruz *et al.*, 2017). The World Health Organization (WHO) defines traditional medicine as any sum of total knowledge, skill and practices which comes from a particular culture through theories, beliefs, and experiences in health-related conditions. Traditional medicine is identical to modern medicine in that it aims to provide maintenance of health which also paves way for improvement of life quality (Chaudhary & Singh, 2011). In fact, traditional medicine has long existed before modern medicine hundreds of years earlier and has been proved to contribute fruitful finding to modern medicine such as the emergence of the first pharmacologically active compound morphine in the year 1805 (Yuan *et al.*, 2016). *N. sativa* is a medicinal plant that has been studied for its antimicrobial and anti-inflammatory properties (Ahmad *et al.*, 2013; Al-Ghamdi, 2001; Forouzanfar *et al.*, 2014). In this review, the therapeutic role of

N. sativa as a comprehensive bioactive natural product that can be an alternative treatment modality for periodontitis is discussed, particularly via promoting remineralization of the tooth and alveolar bone. A detailed emphasis is given on the effects of *N. sativa* towards genes involved in remineralization.

Methodology

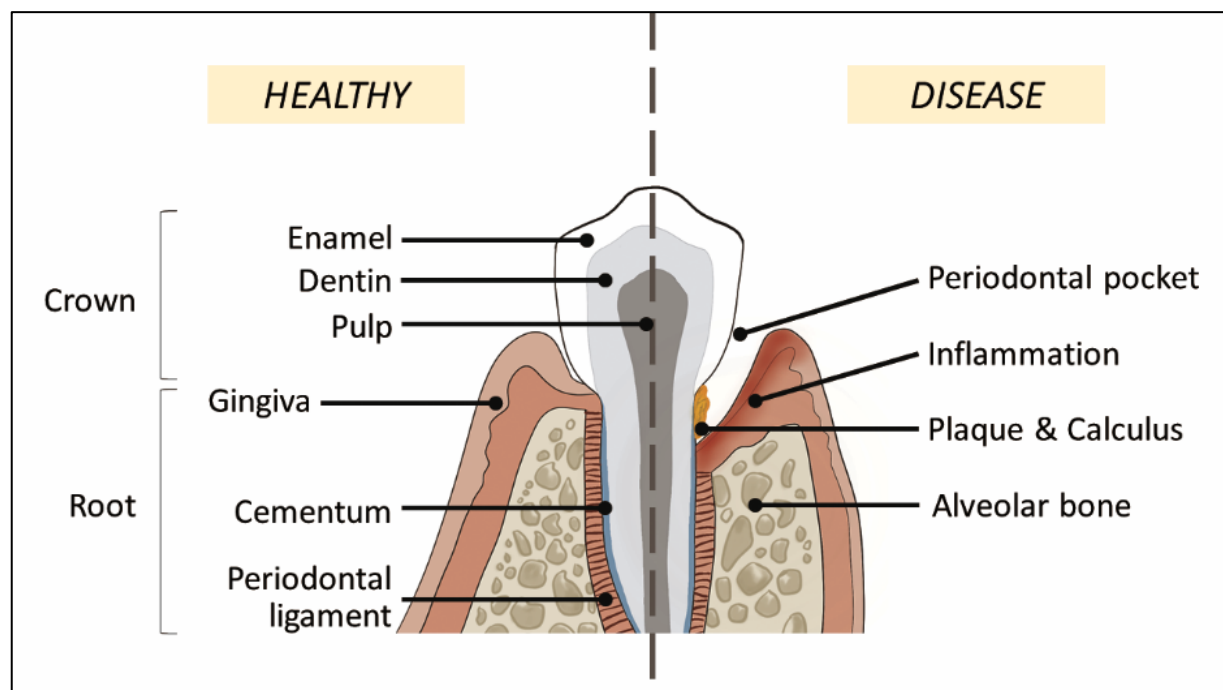
This article adopts the narrative review approach in which relevant articles written in English were retrieved from Scopus, Science Direct and Google Scholar database. Articles and books from the year 1991 to 2020 were selected to include fundamental knowledge on teeth and its development as well as recent studies related to *N. sativa*, mineralization and its impact towards periodontitis. In the light of genetic impact of *N. sativa* on alkaline phosphatase expression, we included keyword search of “*Nigella sativa* and its constituents’ therapeutic properties”, “mineralization”, “alveolar bone remodelling”, “periodontal ligament gene expression”, “ALP gene expression” and “*N. sativa* genetic impact”. Six themes were covered under the following subtopics: (1) Teeth mineralization, demineralization, and remineralization, (2) Periodontal tissue, (3) Gene expression of the periodontal ligament, (4) *N. sativa* and its therapeutic properties on dental health, (5) *N. sativa* effects on teeth mineralization and ALP expression, (6) *N. sativa* additive effect. This review also covers the possible use of *N. sativa* coupled with antibiotics to enhance its effect in treatment.

Teeth mineralization, demineralization, and remineralization

Human teeth are structurally formed by three layers which include the enamel, pulp-dentin complex, and cementum (Figure 1). Enamel is the outermost whitish calcified layer that makes up the crown structure and teeth root. Pulp-dentin complex is present in the inner layer consisting of dentin as the immediate layer after enamel and pulp having all the capillaries supplying blood

and nerve to the teeth. Cementum covers the root surface to maintain dental and periodontal attachment (Boskey, 2007). For dentin and cementum, almost 90% of these structures are made up from collagen,

specifically from collagen type I. On the other hand, nearly 90% of the enamel structure is composed of non-collagenous protein (Margolis *et al.*, 2014).



According to Caruso *et al.* (2016), mineralization starts at the dentin structure through a process known as dentinogenesis. This process is further subdivided into three distinct stages dependent on the site of mineralization. The first stage is vesicle derived mineralization that occurs in the mantle or the outer layer of dentin. Within odontoblastic processes, matrix vesicles (MVs) are vesicular bodies found in dentin and are present in the extracellular environment during the mantle dentine mineralization process (Garcés-Ortíz, 2013). MVs are formed via budding events of odontoblasts that reside in the basement membrane. The vesicles produced would then provide initial mineralization components, including enzymes required for dentin (Golub, 2009). The second stage is the extracellular matrix (ECM) molecule-derived mineralization. This process appears when odontoblasts secrete type I collagen and proteoglycans, particularly chondroitin sulphate which functions to attract calcium ions and acts as a transporter

for mineral from cells into the ECM. These components make up the matrix of dentin and induce differentiation of pre-dentin to dentin. Some non-collagenous proteins (NCPs) are also being secreted influencing the nucleation and also inhibition of mineralization. Finally, the third stage is blood-serum-derived mineralization where there is a passive deposition of proteoglycans, lipid and ECM secreted from odontoblasts or from the dentinal lymph. These depositions give rise to the formation of a thin amorphous matrix in the peritubular dentin (Goldberg *et al.*, 2011). In the matrix-derived mineralization, type I collagen is the main component of dentin being incorporated equivalent to more than half of mineral contents while there are some parts of NCP present acting as a promoter or an inhibitor. These proteins inhibit immature mineralization of dentin until proper orientation and arrangement of collagen fibril are achieved (Van Der Rest & Garrone, 1991).

After the mineralization of dentin, the enamel structure undergoes a similar process by the action of ameloblasts cells which shares a common origin with odontoblasts from mesenchymal stem cells (Moradian-Oldak, 2012). These cells secrete enamel matrix protein and proteinases, which contribute to the immediate mineralization of enamel. Enamel crystal ribbon is initially formed within the mineralized dentin surface along with the secreted enamel matrix proteins that grow away outwards from the dentin. Consequently, full-scale enamel thickness is produced, causing ameloblasts to become protein-resorbing cells to remove enamel matrix proteins. Due to the removal, the structure is compensated by minerals and water making up the structure with approximately 95% mineral contents. Further calcification process of teeth occurs as calcium is transferred from the blood into the enamel matrix where they are deposited (Abou Neel *et al.*, 2016).

Under normal circumstances, demineralization of the teeth occurs all the time, particularly when subjected to acidic contents from the diet as well as from normal oral microbiota (Abou Neel *et al.*, 2016). Demineralization of the tooth indicates minerals wearing off from the enamel and dentin, which can cause the teeth to become porous. Nevertheless, the number of minerals being washed away are small and do not affect the deeper hard tissues. Saliva involves in the mechanism that the acid produced from diets or normal flora in the oral cavity. However, periodontitis, an inflammation event affecting the periodontium of the tooth, largely promotes demineralization of the teeth (Gasner & Schure, 2020). Untreated and prolonged gingivitis leads to the extensive growth of bacterial biofilm deep beyond the gingival margin. This condition causes the deepening of the periodontal pocket (Figure 1). The condition worsens following the destruction of the periodontium (consisting of the periodontal ligament, cementum, gingiva, and alveolar bone) whereby demineralization that extends up to the alveolar bone, further

weakens tooth attachment and ultimately causing tooth loss (Larsen & Fieehn, 2017). Remineralization can be promoted by keeping away the bacterial plaque from depositing in the teeth through proper brushing and flossing the teeth. Another way is to use fluoride contained toothpaste as it strengthens and induce remineralization. Vitamin D and natural products may also help in promoting remineralization (Philip, 2019). Factors that promote remineralization includes the time-dependent exposure of tooth surface to the calcium, phosphate and fluoride brought by the saliva (Garcia-Godoy & Hicks, 2008).

Periodontal tissue

Periodontal tissue is located between the cementum and the alveolar bone, as depicted in Figure 1. It is characterized by the dense fibrous connective tissue that acts as a support medium and attachment for the teeth to its socket. The major component of periodontal tissue essential for normal teeth function is the periodontal ligament (PDL) which contains a heterogeneous cell population that are fibroblastic in nature as well as cells that contribute to mineralized tissue formation (Dean, 2017; Maeda *et al.*, 2011; Marchesan *et al.*, 2011).

This remodelling capacity allows it to differentiate into specialized tissues which are bone, cementum, and connective tissue fibres. Meanwhile, the undifferentiated cells present around the blood vessels, namely ectomesenchymal cells, have the capability to differentiate into cementoblasts, osteoblasts and fibroblasts (Caetano-Lopes *et al.*, 2007).

The PDL functions as a supportive structure that strengthens the teeth attached to the alveolar bone socket (Dean, 2017). The principal fibres of the periodontal ligament are the key element that serves as a bridge that unites the root cementum with the bone by strong fibrous connection (Matsuo & Takahashi, 2002). Besides, the periodontal ligament, particularly the principal fibres, also acts as an attachment medium and shock-absorber that can withstand heavy

occlusal force during mastication (Li *et al.*, 2018). In addition, blood-origin macrophage also supplies the area with multinucleated cells which are osteoclasts and odontoclasts. These two cells are involved in the process of bone and tooth resorption (Dean, 2017).

Other functions of the PDL are sensory and nutritive. Nerve ends are deposited inside the periodontal ligament, providing receptors for pain and pressure (Dean, 2017). The periodontal ligament also possesses well-vascularization which arises from the dental arteries. This vascularization provides nutrient for the cell surrounding vitality (Dean, 2017).

Gene expression of the periodontal ligament

According to Choi *et al.* (2011), the gene expression involved in osteogenic events of PDL follows a temporal sequence beginning from the proliferation stage, subsequently the bone matrix formation and maturation stage, and finally the mineralization stage. During the proliferation stage, *c-myc*, a cell growth-regulated gene expressed in PDL encodes proteins that induce proliferation by functioning as transactivation factors. After cell proliferation is downregulated, alkaline phosphatase (*ALP*) mRNA expression is elevated to enter the cell differentiation stage. *ALP* is one of the biomarkers that influence PDL cell differentiation into osteoblastic cells (Choi *et al.*, 2011). As the bone matrix continues to develop, *ALP* expression elevates, thus making the protein as one of the markers for osteoblastic cells.

Human *ALP* is categorized into four subtypes which are tissue nonspecific, intestinal, placenta and germ cells. Tissue nonspecific *ALP* (*TNAP*) is responsible for bone and teeth mineralization (Millán, 2013). *TNAP* is mainly expressed by chondrocytes, osteoblasts, and odontoblasts (Chaudhary *et al.*, 2016; Hoylaerts *et al.*, 2015). *TNAP* is concentrated in the vesicles which bud off from those cells to supply through vesicle-derived mineralization. During the mineralization process, *TNAP*

activity catalyses the conversion of PPi into Pi . PPi is an inhibitor of hydroxyapatite crystal formation. Meanwhile, Pi is needed to form hydroxyapatite crystal (Orimo, 2010). *ALP* gene is located on chromosome 2q37.1 (Lee *et al.*, 2007). Inhibition of *ALP* gene expression may halt the mineralization process leading to hypomineralization, thus highlighting its crucial role (Orimo & Shimada, 2008).

During PDL differentiation, the *BMP* gene was also expressed, particularly *BMP-2* and *BMP-4*. *BMP-2* and *-4* showed similar expression patterns to *ALP* (Choi *et al.*, 2011). *BMP* is important as a chemoattractant for differentiation of undifferentiated mesenchymal stem cells into areas of bone loss, thus increasing the bone matrix protein. Besides being expressed in the PDL fibroblasts, gingival fibroblasts also express *BMP* and *ALP* (Rahman *et al.*, 2015).

The active expression of *ALP* was followed by the expression of osteocalcin (*OC*). *OC* is a protein that is routinely used as a serum marker for osteoblastic bone formation and currently suggested to demonstrate hormonal roles that extend to involve regulation of whole-body metabolism, cognition, and reproduction (Zoch *et al.*, 2016). The increase in *OC* gene expression in PDL cells was shown to correlate with the formation of bone nodules. At the terminal differentiation of osteoblastic cells, *c-fos* expression was prominent (Choi *et al.*, 2011). *c-fos* is a cell growth-regulated transcription factor that has a specific association with bone tissue (Yamaguchi *et al.*, 2002).

Other than that, in the events of infection or orthodontic tooth movement due to orthodontic force, interleukin-1 (*IL-1*) and interleukin-8 (*IL-8*) are expressed in PDL, which invoke an early inflammatory response and osteoclastic bone resorption (Lee *et al.*, 2013). *IL-8* plays a key role in neutrophils activation and recruitment whereas *IL-1 β* is an important bone resorption cell mediator (Tuncer *et al.*, 2005). Following traumatic condition, necrotic cells are formed, and the nearest

healthy ligament cell will aid in repair. However, excessive chemokine response may develop chronic hyperinflammatory lesion leading to periodontal ligament and alveolar bone loss (Huang *et al.*, 1999; Nibali *et al.*, 2013). It is evident that IL-1 is essential in the pathogenesis of periodontitis. Therefore, the genes encoding IL-1 production had been regarded as a potential predictor of periodontal disease progression (Grigoriadou *et al.*, 2010).

***Nigella sativa* (*N. sativa*) and its therapeutic properties on dental health**

N. sativa or commonly known as black seed or *Habbatus sauda'* is a medicinal seed that is known for its therapeutic effects. *N. sativa* seed and its extracted oil are used as a therapy due to its wide properties including antimicrobial and anti-inflammatory. The main active compound of *N. sativa* is thymoquinone (30-40%) which is proposed to be responsible for the antimicrobial and anti-inflammatory properties (Ahmad *et al.*, 2013). Thymoquinone (TQ) has been shown to possess antimicrobial effects demonstrated by the growth inhibition of bacteria such as *Staphylococcus aureus* (*S. aureus*) when treated with the active compound. TQ has been demonstrated to be more effective against gram-negative bacteria and that with wide range of antibiotic resistance (Ahmad *et al.*, 2013). Anti-inflammatory effects of TQ are attributed to its ability to inhibit key inflammatory cytokines such as IL-1 and nuclear factor κ B. Besides TQ, other bioactive constituents of *N. sativa* such as dithymoquinone, thymol, and thymohydroquinone are also being studied for their therapeutic properties (Gholamnezhad *et al.*, 2016).

In relation to oral care, TQ offers a promising therapeutic candidate due to its ability to reduce dental caries and plaque index of teeth (Al-Attas *et al.*, 2016; Angius *et al.*, 2015). TQ is proved to be effective as an inhibitory agent against attachment and colonization of dental microbes such including *Streptococcus mitis* (*S. mitis*) and

Streptococcus mutans (*S. mutans*) (Harzallah *et al.*, 2011). Besides influencing bacterial colonization, TQ also exerts both antioxidant and anti-inflammatory effects. Periodontitis can be caused by oxidative stress in which reactive oxygen species (ROS) increase beyond the tolerable limit of antioxidant capabilities. Diminished antioxidant scavenging activities lead to periodontal tissue destruction through lipid peroxidation. In the case of *Porphyromonas gingivalis* (*P. gingivalis*) induced inflammation through its lipopolysaccharide (LPS), which causes the excessive formation of mitochondrial ROS. TQ, with its antioxidant properties, is suggested to induce ROS scavenging activity, thus reducing the further breakdown of periodontal tissues (Bullon *et al.*, 2014). Thus, this impedes the action of ROS that disrupts with the osteoclastic activity. TQ has been shown to reduce alveolar bone loss, infiltration of inflammatory cells and osteoclasts activities while improving osteoblastic differentiation on periodontium (Al-Ghamdi, 2001). Therefore, the coupled effect of the antimicrobial and anti-oxidative property of TQ is suggested to be a suitable candidate in the formulation of treatments in the management of periodontitis.

***N. sativa* effects on teeth mineralization and ALP expression**

N. sativa can induce anti-osteoporotic conditions due to its antioxidant and anti-inflammatory properties that prevent the destruction of bone components (Rukshar & Neha, 2013). These properties are beneficial in treating periodontitis. *N. sativa* has been reported to induce the expression of ALP which is linked to the mineralized-tissue-forming cell differentiation as well as increasing bone nodule formation (Wirries *et al.*, 2013). Other genes such as OC, osteopontin (OPN) and BMP gene are also stimulated by *N. sativa* specifically by its active component the thymoquinone (Islam *et al.*; 2016Wirries *et al.*, 2013). BMP genes, particularly BMP-2, increases its expression through the ERK pathway, which is known to involve in bone formation and osteoblast

differentiation (Jun *et al.*, 2010; Su *et al.*, 2010).

Alveolar bone loss is a key feature of periodontitis progression, and this condition is associated with enhanced osteoclast formation. Periodontitis induces osteoclast formation by increasing the differentiation of quiescent osteoclast precursors into osteoclasts (Lee *et al.*, 2015). Thummuri *et al.* (2015) highlighted that TQ could reduce osteoclastogenic activity exerted by receptor activator of nuclear factor kappa B ligand (RANKL) through inhibition of MAPK and NF-kB signalling pathway. NF-kB and MAPK are involved in osteoclast differentiation alongside with JUN and p38. Through activation of the MAPK pathway, *c-fos* is induced, thus increasing the osteoclastogenesis, which leads to bone resorption (Boyle *et al.*, 2003; Lee *et al.*, 2009). Meanwhile, inhibition of TQ halts both the activation of MAPK and p38, leading to decreased cellular differentiation of osteoclasts, in turn reducing its downstream effects.

The destructive effects of periodontitis are also incited by subgingival plaque microflora and LPS derived from pathogens (Hienz *et al.*, 2015). TQ could also prevent osteoclastogenic activity by LPS through the MAPK, NF-kB and TLR signalling pathway (Thummuri *et al.*, 2015). This is complementary to the antimicrobial properties of TQ, which is beneficial to the suppression of bacterial biofilm formation.

***N. sativa* additive effect**

N. sativa has been reported to possess additive effect in eliminating microbial when combined with doxycycline (Aljabre *et al.*, 2015; Forouzanfar *et al.*, 2014; Halawani, 2009). Doxycycline is an antibiotic in tetracycline class that has frequently been used for periodontitis treatment (Garret *et al.*, 1999; Kopytynska-Kasperczyk *et al.*, 2015). In fact, doxycycline is semi synthetic and more active compared to other antibiotic in the tetracycline class. Doxycycline is effective in eliminating many bacteria species includes enterococci,

Streptococcus pyogenes, anaerobic, various *Nocardia* spp. and *S. aureus* which is originally resistant to tetracycline (Kogawa & Salgado, 2012). Doxycycline is bacteriostatic, exerting antibacterial action by inhibiting bacterial growth and prevent the bacteria from reproducing (Loree & Lappin, 2019). Doxycycline binds to the 30S ribosomal subunit in protein translation process to stop amino acid from being linked and inhibit the bacterial protein synthesis (Kogawa & Salgado, 2012; Raval *et al.*, 2018). Besides, it has anti-collagenase properties that can help to protect and maintain the collagen composition on the site of treatment (Raval *et al.*, 2018) while at the same time eradicating the pathogenic bacteria. On the downside, doxycycline must be administered at proper dose as gastrointestinal effect will arise from the usage of the antibiotic such as stomach irritation, ulceration (Raval *et al.*, 2018) and phototoxic reaction (Skidmore *et al.*, 2003). Hence, addition of any compound on top of doxycycline that can buffer the toxic effect can be considered to ensure an effective treatment.

Conclusion

Traditional medicine had used medicinal herbs as therapeutic agents to cure oral diseases. It has been suggested that *N. sativa* or black seed have great benefits in improving oral health. Evidence of a coupled effect (antimicrobial and antioxidant) of TQ towards the main pathogenicity of periodontitis further highlights its potential as an alternative treatment modality. Further research is essential to study the optimal dosage of *N. sativa* that is therapeutically effective as well as analysis on the toxicological profile to ensure the safety. Several genes, especially the *ALP* gene, can be a suitable candidate as the downstream target.

N. sativa coupled with tetracycline has an additive effect against pathogenic bacteria. In this light, a possible therapeutic approach is that *N. sativa* can be combined with doxycycline, a type of tetracycline antibiotic to serve as antimicrobial, remineralization

and regeneration agent for remediating cell structures damaged by periodontitis. Nevertheless, the correct formulation is needed to achieve stability for the formulation. Doxycycline can be effective, but without proper dosage quantification and without additional compounds to enhance its efficacy, disease recurrence is inevitable.

Conflict of interest

The authors declare no conflict of interest and that the review was conducted in the absence of any financial relationships that could be construed as a potential conflict of interest.

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