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Diabetic Foot Ulcer Healing Potential Mechanism from *Azadirachta indica* (Neem) Leaves

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ABSTRACT

Diabetic wound is a major problem in the world health community. The incidence, morbidity and mortality are increasing, due to its major complications including the diabetes related foot disease. Improvement and innovations in the wound care armamentarium has been developed including from natural sources, but only relatively small portion can be applied, especially in developing countries. The lack of justifying comprehensive evidence of basic bio-active component and mechanism of action halting the progress to clinical applications. Natural wound product such as Neem (*Azadirachta indica*) has gaining interest in laboratory and clinical field. Narrative review of the literatures was done, in effort to elucidate the mechanism of action for applications in diabetic wound. Contributing into the current knowledge and gathering data of the main bioactive compounds and building evidence to support the applications of any potential form of neem leaves for the management diabetic wounds in clinical setting.

Keywords: Diabetic foot ulcer, Wound healing, Neem, *Azadirachta indica*.

INTRODUCTION

Diabetes Mellitus (DM) and its foot complications is overwhelming global health. The rise of epidemiological parameters of 2014 prevalence to more than 400 million cases, significantly adding more numbers to the rate of morbidity and mortality [1]. The risk of DM patients for contracting diabetic foot ulcer (DFU) is in the range of 19-34%. Re-occurrence of ulcer is expected in 6 out of 10 patients in 3 years. Half of the DFU will be infected and increasing the risk of limb loss. Moderate to severe infection especially osteomyelitis will be resulting in 20% lower extremity amputation (LEA) [2]. Amputation related to DM complications is done every 20 seconds, with the ulcer as an initiating event in 85% LEA. Individual with DFU will have 2,5 times higher risk of mortality in 5 years, compared to DM individuals without DFU. Impact on healthcare cost is massive with loss of productive earnings and cost to carers. Data from low-resources country showed higher proportion of wound severity and amputation prevalence [3]. This will increase the spending of the country and personal funds, and negatively impact the economy. Adding to all problems, diabetic foot wounds is a major contributor to the global disability and worsen the quality of life, mainly in geriatric patients [4].

To overcome hard-to-heal wound and infection-prone ulcer demands effective and efficient therapy to facilitate healing and preventing or eradicating infectious agent. Oxidative stress induced by hyperglycaemia damaging important cells such as neurons (neuropathy), endothelial cells of blood vessels (angiopathy), blunting the diabetic host immune response to infections and maintaining pro-inflammatory state in wound environment, thus halting the transition to proliferative phase and epithelialization. These highlight the importance of wound therapy armamentarium that is safe and effective to shift the inflammatory state to proliferative stage, eradicating pathogens, efficiently protecting the tissue from free radicals, easy to apply, and at lower cost to produce [5]. Plants with therapeutic potential such as Neem (*Azadirachta indica*) tree may be the answer to the problems mentioned above. Neem has widespread use and inherits from ancient era to this days. That is why, we need to explore the scientific literature and try to make this review about healing potential from neem to cure diabetic and other chronic wounds.

MATERIALS AND METHODS

A literature search was conducted in electronic databases including PubMed, EMbase, Cochrane and Google Scholar. The following search terms were used: ("neem leaves" OR "*Azadirachta indica*") AND ("chronic wounds" OR "wound healing" OR "wound closure"). The search was limited to articles published in English from the year 2000 to 2023. We include studies investigating the bioactive composition of neem leaves and studies evaluating the effects of Neem leaves on chronic wound healing including in vitro in vivo, and clinical studies. We exclude non-English articles and studies not focusing on chronic wound

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healing or Neem leaves.

Pathophysiology of Diabetic Wound

The wound healing process is a complex process that involves coordination and interaction between various types of body cells and inflammatory mediators. Injured cells in the wound area will send signals in the form of cytokines and growth factors to initiate the process of skin tissue restoration. This recovery process occurs based on the extent of tissue damage and the presence of foreign antigens that trigger the activation of acute inflammatory cells [6]. Platelet fragments play their first role by initiating the coagulation cascade after being activated by endothelium, to stop bleeding (hemostasis). Activated platelets, keratinocytes, and damaged or hypoxic endothelium release pro-inflammatory cytokines such as IL1b, TNFa, IL 6, and IL 8 that attract and recruit neutrophil and macrophage cells to the wound area and produce growth factors such as platelet-derived growth factors (PDGF), keratinocyte growth factors (KGF), transforming growth factors (TGF b), and fibroblast growth factors (FGF) that are important for inflammation and proliferation phases [6]. The entry of acute inflammatory cells functions as a cleaner for the wound area from bacteria, foreign objects, and dead tissue so that it is ready for further processes. Phagocytosis and cytotoxicity of neutrophils and macrophages depend on how much pro-inflammatory stimulus is produced in the wound area. Once the wound area is relatively clean, and the environment begins to supply blood flow carrying oxygen and nutrients, then the balance will shift towards anti-inflammatory with the release of anti-inflammatory cytokines such as IL-4 and IL-10, which cause a decrease in pro-inflammatory cytokines, and the wound healing process enters the proliferation phase [7].

Normal progression to restore skin integrity is altered and delayed in diabetic person. Especially with the establishment of complications of neuropathy, angiopathy and kidney failure. Ulcer will develop in denervated feet because of predisposition to injury caused by intrinsic muscle weakness and foot deformity, loss of protective sensation, and decreased moisture [2]. Foot deformity led to abnormal pressure to the plantar side of foot, and callus formation. Chronic abnormal pressure in dry skin and above the bony prominence, will cause subcutaneous skin haemorrhage, and disintegrity. Normal neutrophil and macrophages influx decreased, since the hypoxic and hypercoagulation condition and impaired production of mediators from keratinocytes, platelets, and endothel. Subsequent invasion from nearby bacteria with the release of toxins, further destroying tissues and lead to necrosis of the cells [8].

Oxidative stress induced by hyperglycaemia, impairs haemostasis and inflammatory response [9]. Reactive oxygen species produced by the hyperglycaemia is the results of the polyol pathway and protein kinase C activation, increase hexosamine pathway, diacylglycerol pathway, nitric oxide blocking and glucose autoxidation. These pro-inflammatory metabolism pathways increase the production of oxidant and toxic substance such as advanced glycation end-products (AGEs), further stimulating cells to produce cytokine and growth factors such as TNF-alfa, TGF B1, ICAM-1. VCAM-1, CTGF, and PAI-1 [10]. Persistence inflammatory phase thus delayed the progress of healing. Dysfunction of macrophage polarization and neutrofil function, impaired keratinocytes and fibroblast migration caused by deficient of the growth factors needed. Narrowing of the blood vessels and glycation of haemoglobin causes deficient nutrition supply and low oxygen concentration. This condition deformed proteins and lead to the productions of unfolded proteins (UPR) in response to ischemic stress. Changes in cells miRNA levels also contribute to the halted epithelization, angiogenesis, and migration of fibroblast and keratinocytes. Formation extracellular matrix and granulation tissue is slowed and stopped because of inhibition of fibroblast and new vessel proliferations, dis-balance between MMP and TIMPs resulting in destruction of matrix proteins and growth factors [9].

Bioactive Composition of Neem Leaves

Herbal products have been used traditionally for long time and bringing high contributions in the history of health of the world population. Ancient medicine system from Ayurveda, Unani, and Chinese are being explored to uncover the potential drug candidates to be used in modern medicine. Synthetic drugs have many unwanted effects, costly and sophisticated in the production. Because of these disadvantages, medicinal plants are attractive alternatives for their relatively easy to obtain, low toxicity and comparable efficacy especially to treat wound [11,12].

The neem tree (*Azadirachta indica*) is a tropical medicinal tree that originated from Indian sub-continent and has been spread worldwide. Famous for the epithet "Tree of the 21st Century" from United Nations, this tree, and its parts, has long history in treating ailments and been used for personal hygiene, infections and inflammation problems, metabolic and cancer. has several contents that have anti-microbial and anti-inflammatory effects [13]. Phytochemical research shows that various parts of neem contain mainly flavonoids, limonoids, tannins, saponin, gallic acid and triterpenes such as: azadirachtin, meliacin, gedunin, nimbidin, gedunin, salanin, nimbin, nimbidol, quercetin. These contents have antibacterial, anti-inflammatory, and antioxidant properties that can help accelerate wound healing [14].

In particular, the Nimbin (triterpenes) in neem have been shown to have antipyretic, fungicidal, antihistamine, and antiseptic properties. Nimbin can also reduce the damage caused by production of reactive oxygen species, as it has antioxidant capacity. In addition, neem flavonoids content function as inhibitors of prostaglandin biosynthesis, endoperoxides, and enzymes such as protein kinases and phosphodiesterases, all of which can reduce inflammation. Polyphenols such as Quercetin and β-sitosterol were the first flavonoids purified from fresh neem leaves and are also known to have antifungal and antibacterial activities [15]. Neem oil extract is the most used form. In-depth phytochemical analysis has confirmed the presence of high amounts of triterpenes, flavonoids, and saponins, while other components such as catechins, appear to be present in lower amounts. Other metabolites found in neem extracts are: limonoids, tannins, alkaloids, terpenoids, reducing sugars, catechins, sterols, and gallic acid [16].

Neem has a complex of various constituents, and such types of ingredients play a role in disease management through modulation of various genetic pathways and other activities. The most important constituent is azadirachtin and others include triterpenes (nimbin) tannins (epoxy-azadiradione), Limonoids (nimbolide), nimbinate, gedunin, salanin, and flavonoids (quercetin) [15]. One glycoprotein

(Neem leaf glycoprotein/NLGP) leaves also shown immune-modulating capability capable of restricting tumour growth [17].

Composition analysis showed variable bio-active compound according to the type of the extract. Aqueous extract showed high levels of saponins, tannins (epoxy-azadiradione, nimbin, azadirachtin) and glycosides in aqueous extracts, while methanoic extracts showed high levels of alkaloids, tannins, and flavonoid [18]. Other substance such as glycosides nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, and n-hexacosanol, benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, hydroxyazadiradione, and nimbioland in non-methanoic leaf extract. Neem also has high proline content, which is a current treatment for neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, type 2 diabetes mellitus and polycythem [19,20].

Possible Mechanism of Neem Leaves on Chronic Wound Healing

Several studies have demonstrated the potential of Neem leaf extracts in promoting wound healing. Neem leaf extracts have shown positive effects on various aspects of the wound healing process, including anti-microbial, promoting angiogenesis, collagen synthesis, re-epithelialization, and wound contraction.

The mechanism of chemical contents in *Azadirachta indica* in wound healing is through several ways (Figure 1). First, the antibacterial properties of the contents in neem can help prevent infection in wounds which can slow down the healing process. Nimbin and azadirachtin content has been proven to have a strong antibacterial effect against various types of bacteria that are often associated with wound infections. Ethanol extract of neem leaves had been shown capable of inhibiting gram positive and negative bacteria including Strains of Staphylococcus, Enterococcus, Klebsiella and Pseudomonas aeruginosa. Anti-bacterial properties of ethanol extract from neem leaves also showed inhibition of MRSA strain. Other animal studies show the potential of neem extract to accelerate diabetic ulcer wound healing because it is antiseptic and has anti-inflammatory and antioxidant properties [20-22].

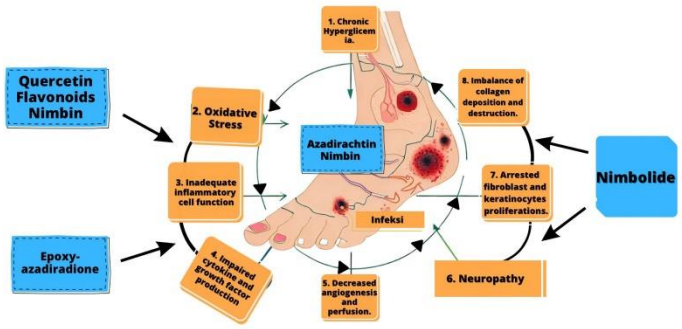

Figure 1: Mechanism of neem bioactive compounds in diabetic foot ulcer healing

Main component of neem leaves extract proved to be anti-inflammatory, and antioxidant are the triterpene nimbin, nimbidin, flavonoids, NLGP, epoxyazadiradione and other limonoids. These are achieved by interfering with several pathways such as inhibits the pro-inflammatory mediator's production, antioxidant and modulating the activity of immune cells especially the neutrophils and macrophages. Neem extract especially methanol, has highest flavonoid and phenol content compared to aqueous extract [23]. The activity is proven in several model of inflammation in rats. Neem extract reduces the myeloperoxidase, xanthine oxidase as a marker of inflammation, and also increasing the body's natural antioxidant system such as increasing glutathione, and superoxide dismutase (SOD), nitric oxide (NO) and reduced expression ERK1/2 [24]. Rich flavonoids content can inhibit prostaglandin, cyclooxygenase, endoperoxidases, and phosphodiesterase biosynthesis, thus weaken the inflammatory processes [21]. Limonoids in neem had been shown to decrease oedema at 120mg/kg dose with inhibition of TNF alpha and interleukins [25]. Epoxy-azadiradione is another neem limonoids with major action through modulation of macrophage inhibitory factors, blocks its tautomeric activity and the NF-kB translocation, and so preventing the release of pro-inflammatory cytokines such as IL-1a, IL-6 and TNF -alpha [26].

Recent studies by Maan et al. showed that *Azadirachta indica* stem bark extract can increase collagen production and proliferation of regenerating cells. This results in significantly faster wound contraction, and stronger wound tensile strength in excision and incision wound mice models [27]. Formulation of neem seed oil emulsified with an alkyl polyglucoside as a protectant increase wound breaking strength in incision model, significantly accelerating wound contraction in excision model 95% in day 16th with a re-epithelialization period of 10.21 with 2.31 SD. From microscopic evaluation, treated sample with neem showed faster development of dermis/epidermis and collagen and simultaneously inhibit MRSA colony showing anti-bacterial and fibroblast/keratinocytes proliferation influence of neem extract [28]. Preliminary studies and case reports of wound care using neem irrigation fluid have accelerated wound healing and have not caused harmful side effects in diabetic foot patients with ulcers [29,30].

CONCLUSION

Neem (*Azadirachta indica*) leaves possess a rich composition of bioactive compounds that hold potential for use as a treatment for chronic. The antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory properties of Neem leaves contribute to their effectiveness in promoting wound healing. Neem leaf-based formulations have shown promise in accelerating the wound healing process and reducing complications. However, further research, including well-designed clinical trials, is needed to validate these findings, determine optimal dosages, and establish standardized Neem leaf-based interventions for chronic wound management.

Conflict of Interest

The authors declare no conflicts of interest.

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