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Micronutrients and Natural Compounds Status and Their Effects on Wound Healing in the Diabetic Foot Ulcer

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Abstract

The diabetic foot ulcer (DFU) is an invariably common complication of diabetes mellitus, it is also a significant cause of amputation as well as extended hospitalization. As most patients with DFU suffer from malnutrition, which has been related to improper metabolic micronutrients status, alterations can affect impaired wound healing process. Micronutrients and herbal remedies applications present a wide range of health advantages to patients with DFU. The purpose of this review is to provide current evidence on the potential effect of dietary supplementations such as vitamins A, C, D, E, magnesium, zinc, copper, iron, boron, and such naturally occurring compounds as *Aloe vera*, Naringin, and Radix Astragali (RA) and Radix Rehmanniae (RR) in the administration of lower extremity wounds, especially in DFU, and to present some insights for applications in the treatment of DFU patients in the future.

Keywords

micronutrients, diabetic foot ulcer, wound healing, herbal remedies

Nutrition has been recognized as an important element in helping wounds to heal and the improvement of arterial disease, and it plays a significant role in the process.[1-3] Micronutrients also play a vital role in the form of trace elements and vitamins, since they influence the course followed by the healing wound.[4] When supplementary micronutrients are administered, the risk of developing pressure wounds may be reduced. A randomized control trial showed that giving nutritional supplement reduced the incidence of pressure ulcers in bedbound patients.[5] When wounds heal, the process involves both hypermetabolic and catabolic states. Catabolism may be reduced by nutrients, limiting its effects thereby leading to the anabolic state.[6,7] In the healing process, it is therefore beneficial to provide trace elements such as magnesium, zinc, iron and boron, vitamins A, C, D, E or herbal remedies. This will also apply in the case of wounds to the lower extremities. Nutritional assessment is a complex task usually carried out by trained dietitians and/or suitably trained physicians to provide detailed account of the nutritional status of a patient. The aim of this review is to provide current evidence on the potential effect of intake of micronutrients and natural compounds supplementation for the management in diabetic foot.

Search Strategy

A literature search was performed using such electronic databases as Scopus, Web of Science, internet searches and other trials registers, including Science Direct and Clinical Trials.gov. Some of the appropriate keywords used included dietary, micronutrients, natural compounds, supplementation, wound healing, and diabetic foot ulcer (DFU). All published data from 1980 to March 31, 2017 relevant to the effect of dietary micronutrients and natural compounds supplementation for patients with diabetic foot were retrieved in the review.

Authors independently selected 38 studies, and extracted the data. In case of an inconsistent decision, all authors discussed the selection to find a unanimous solution. The reference list of each selected article was reviewed for further relevant study.

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Vitamins

Vitamin D. Many important biological and physiological processes are influenced by vitamin D.[8] When vitamin D is not present in sufficient quantities, its absence will affect dermal fibroblast-mediated tissue repair since this is normally promoted by the transforming growth factor beta 1 (TGFβ1). If this occurs, the healing of the wound will take much longer than usual. In contrast, when vitamin D levels are high, wound healing is accelerated even if TGFβ1 levels are low. For this reason, it is useful to support wound healing with vitamin D supplements, which may also have the effect of reducing fibrosis.[9]

Razzaghi and colleagues[10] studied diabetic patients with foot ulcers and reported that 50 000 IU vitamin D supplements taken at 2-week intervals for a period of 3 months provided benefits in terms of healing through the enhanced glycemic control. Among the outcomes was an improvement in inflammation and oxidative stress.[10] The lower levels of parathyroid hormone production that resulted from vitamin D supplementation can limit the production of C-reactive protein (CRP), which would contributes to inflammation,[11] and can also limit the generation of the reactive oxygen species, which would lead to oxidative stress.[10] A recent study reported that cytokine is also a significant factor in infection, and patients with DFUs exhibited higher concentrations of inflammatory cytokines. Vitamin D deficiencies can exacerbate this scenario since the outcome can be heightened levels of inflammatory cytokines. It is therefore recommended that a 25-hydroxyvitamin D <25 nmol/L supplements be administered to diabetes mellitus patients experiencing foot infections.[12]

Vitamin E. Vitamin E has a history of use in the treatment of skin as well as wound healing going back more than half a century. The category encompasses tocopherols and tocotrienols, and has been shown to be effective in supporting wound healing since it works as the primary lipid soluble antioxidant. Musalmah and colleagues[13] studied the efficacy of α-tocopherol as an antioxidant when applied to wound closure in normal and streptozotocin-induced diabetic rats. The rats were separated into 2 groups; control rats and streptozotocin-induced diabetic rats, and then each category was subdivided into a group receiving treatment and a group not treated. The treatment was a daily dose of 200 mg/kg bodyweight α-tocopherols, and the results revealed a reduction in plasma malondialdehyde levels in the treatment group along with heightened levels of glutathione peroxidase activity, which served to speed the closure of wounds in these rats ($P < .05$).[13]

Jain and Jain[14] examined the activity of vitamin E in preventing diabetic complications from developing and progressing. In the test group, it was reported that 3 key measures were all significantly lowered: postprandial blood sugar (PPBS), total cholesterol (TC), and diastolic blood pressure (DBP). This showed that vitamin E was able to halt the progression of the condition and also slowed the onset of complications, including foot ulcers, cardiovascular disease, and retinopathy.[14]

The problems in wound healing can be examined using diabetic mice through consideration of heightened lipid peroxidation. Galeano and co-workers[15] explained that Raxofelast, a form of vitamin E (IRFI 016; 2,3-dihydro-5-hydroxy-4,6,7-trimethyl-2-benzofuranacetic acid), can serve as a scavenger of reactive oxygen species, which can thus inhibit the lipid peroxidation, and cause damage to capillary permeability, endothelial cells, and fibroblast and collagen metabolism. Raxofelast can prevent lipid peroxidation in the polyunsaturated fatty acids of the membranes through its angiogenic activity. Furthermore, it is also reported to promote the generation of fibroblasts and keratinocytes.[15]

Vitamin C. Vitamin C, or ascorbic acid, plays the role of a reducing agent and thus has an effect in various body tissues. It is active in reproduction, immunity, growth, and reactions to infection. It is a key ingredient in the synthesis of collagen for the skin and is a necessary component in the formation of cartilage. If vitamin C levels are inadequate, wound healing can be adversely affected and the risk of infection is increased. One slight problem for humans is that ascorbic acid cannot be directly synthesized because this would require gulonolactone oxidase enzyme to be available. Hence, it is necessary to take vitamin C in the fruit, vegetable, or tablet form.[16]

Many studies investigated the role of vitamin C in wound healing and found that vitamin C can be associated with antioxidant activity, collagen synthesis, and cellular apoptosis. In the inflammatory phase, it is necessary to have vitamin C for neutrophil apoptosis to occur, while the proliferative phase requires vitamin C for the synthesis, maturation, secretion, and degradation of collagen.[17-19]

A recent study demonstrated that use of maltodextrin/ascorbic acid for treatment through a preclinical model involving in vitro healing in wound healing.[20] The study was also applied to in vivo venous leg ulcers. The in vitro treatments worked in the same way as the in vivo cases, with the addition of maltodextrin/ascorbic acid serving to promote the levels of TGFβ1, as well as MMP-1 (matrix metalloproteinase-1) expression and gelatinase activity. At the same time, TIMP-1 (tissue inhibitor of MMP-1) was reduced, leading to repair of the monolayer through heightened collagen turnover. In addition, as the level of gelatinolytic activity rose, this resulted in the repair of the fibroblast monolayer. Assessment of in vivo cases revealed that patients who received the maltodextrin/ascorbic acid treatment at 12 weeks showed significantly greater improvement in cellular biomarkers and tissue morphology in comparison with the control groups, while wound healing was enhanced by a factor of 3.7 times ($P = .034$). The results suggest, therefore, that maltodextrin/ascorbic acid is a suitable treatment to improve the healing of chronic wounds.

Vitamin C can moderate several phases of wound healing, including inflammation, proliferation, and remodeling, and accordingly, it is confirmed to support the overall process. Vitamin C supplements help complete the inflammatory phase, and when there is sufficient vitamin C circulating in the body this will serve to stimulate fibroblast migration along with matrix deposition and neovascularization as the transcript levels of HO-1, TGFβ, CTGF (connective tissue growth factor), and VEGF (vascular and endothelial growth factor) become extenuated. Furthermore, vitamin C can support the healing of wounds via pleiotropic mechanisms that occur in the metabolism of collagen.[21] It is also understood that vitamin C supplementation can be effective in maintaining the vitamin levels in plasma and tissue, and this is important because these vitamin levels normally fall significantly when they are required to support wound healing.

Vitamin A. Vitamin A, also known as retinal, is necessary for the formation of bone, cellular differentiation, and the immune system. It also promotes wound healing physiologically, and its absence can be detrimental to the process.[22]

It was found that vitamin A can improve the initial inflammation of a wound site by boosting monocytes and macrophages and moderating the action of collagenase. It can also serve to promote epithelial cell differentiation while acting as a stimulant on the immune system.[23]

Meanwhile, vitamin A plays an important role in the skin epidermis, promoting both growth and differentiation. When vitamin A supplementation is administered, the outcomes can include improved macrophage activity and synthesis of collagen. In animals, experimentation has shown that vitamin A boosts epidermal growth factor receptor expression. The recommended supplementation daily dosage of 25 000 IU is aimed to promote wound healing.[7]

Minerals and Trace Elements

Furthermore, vitamins trace elements have been shown to comprise an essential component of the wound healing process in addition to vitamins already discussed. Minerals and trace elements play an important role in DFUs. Frequently, the body requires doses daily in very small amounts for homeostasis, particularly during wound healing. Especially, minerals and trace elements perform as cofactors in many enzymes involved in wound repair. However, inappropriate doses of minerals and trace elements, dietary deficiency or dietary excess, can be harmful.

Magnesium. Magnesium acts as a substantial role in glucose homeostasis and insulin sensitivity. In addition, magnesium acts as a cofactor for many enzymatic reactions. It is also essential for collagen and protein formation and tissue development. In the processing of collagen formation during wound curing, magnesium interacts with adenosine triphosphate (ATP).[24] Low serum magnesium levels are associated with an enhanced risk of developing DFUs. Hypomagnesemia was associated with poor glycemic control, neuropathy, coronary artery diseases, diabetic retinopathy, hypertension, and foot ulcerations.[25] Some studies reported the relationship between serum magnesium depletion and risk factors for the progression of DFUs (odds ratio = 2.9, 95% CI = 1.7-6.8, $P = .01$).[26] The magnesium levels in subjects with diabetics and foot ulcers were lower than those in healthy subjects without foot ulcers ($P < 0.001$).[27] In type 2 diabetes, magnesium deficiency associated with the development of endothelial dysfunction altered insulin function, neuropathy, and abnormal platelet activity.[26,28] Hence, the level of magnesium should be controlled in subjects with type 2 diabetes and foot ulcers.

Zinc. Zinc is an important trace element in various cellular metabolisms. Zinc is a significant part of the human diet it benefits wound healing. Zinc metalloproteinases are involved in DNA and RNA synthesis, protein and collagen synthesis, immune function, cellular proliferation, and wound curing. For the healing process, zinc is also an important cofactor for lysyl oxidase enzymes, which are involved in the cross-linkage of collagen. Tests applied to animals with low serum zinc levels, and also to normal animals,[29] zinc deficiency was reported to delay wound healing.[7] Some research revealed that venous leg ulcers are associated with low zinc levels, particularly if senses of taste or smell are reduced (another possible sign of zinc deficiency), thus zinc supplements may be helpful. Furthermore, Larijani and colleagues[30] demonstrated that serum zinc level was significantly decreased in type 2 diabetes patients with foot ulcers compared to those without foot ulcers. Zinc consumption is 11 mg/d for men and 8 mg/d for women, while zinc supplementation recommendations to improve wound curing is up to 40 mg/d. However, abundant zinc consumption interrupts copper and iron absorption and can cause depletion of these essential minerals.[24] Assessment of mineral contents are needed to confirm that the use of supplementation that will not interfere with trace element homeostasis.

Copper. Copper is a necessary cofactor in protein synthesis and important for collagen formation in wound healing. Thus, copper deficiency may be a cause for impaired wound healing.[7,24]

Iron. Iron acts as a cofactor in collagen synthesis. Iron is associated in wound healing that is required for hydroxylation of two amino acids; proline and lysine.[7] Commonly, anemia and iron depletion are a problem in patients with DFU. Wright and colleagues[31] reported on 27 DFU patients: 14 (51.9%) were anemic, and 2 (7.41%) had severe anemia (Hb <10 g/dL). Takayama and Aoki[32] explained that

lactoferrin, which is a glycoprotein that binds to iron and is secreted from the glandular epithelial cells, serves to support the healing process through its beneficial effect on the initial inflammatory phase. It is able to inhibit excessive immune responses while simultaneously aiding the development of granulation tissue and the process of re-epithelialization. Lactoferrin also promotes the migration of fibroblasts and keratinocytes while boosting the synthesis of collagen.[32]

Boron. Boron is defined as a necessary element for many plants, specially, cell wall structure and the functions of plants. On the other hand, several findings revealed the significance of boron requirements in human nutrition and metabolism, but the mechanism is still unknown. Nevertheless, the effect of boron on diabetic wound curing has not been elucidated so far. A recent study demonstrated that boron derivatives (boric acid and sodium pentaborate pentahydrate [NaB])-containing gel formulation improved rates of wound repairing in streptozotocin-induced diabetic rats and histopathological scores.[33]

Herbal Remedies

Regarding the increased incidence of diabetes, global burden of DFU is dramatically raised and urgently needs to be improved. Traditional medicine has been recommended as an adjunctive therapy for managing DFU. Since impaired wound healing in diabetic patients is complex and involving several factors, natural remedies that combine various therapeutic actions, such as fibroblast-proliferating, antidiabetic, anti-inflammatory, antimicrobial, and antioxidant properties are of interest.

Most founding reports use herbal remedies in a form of wound dressing, but less information is provided for successive oral intervention in acceleration of the wound healing process.

Herbal products that are rich in phenolic compounds, flavonoids, terpenoids, quinones, coumarins, saponins, complex carbohydrates, glycosides or presenting bitter taste, usually exhibit positive effects on controlling diabetic complications. Saponins modulating the activity of enzymes are related to glucose metabolism and improved insulin resistance. Polyphenolic compounds, especially flavonoids, are among the classes of compounds that are focused on the health aspects about their antioxidant properties as well as anti-inflammatory and antidiabetic properties.

Aloe vera. *Aloe vera* is a member of succulent plant in the lily (Liliaceae) family. It is widely naturalized and cultivated in tropical climates for agricultural and medicinal uses. Extracts of the gel are employed in various pharmaceutical preparations for skin, treatment of burns, and for ingestion. Though the gel is considered naturally safe, some people could be allergic to substances in the yellow sap. *A. vera* gel is believed to be a rich source of anti-hyperglycemic and antioxidant compounds, such as saponins, quinones, sterols, and triterpenoids. Another 2 major constituents in *A. vera*, glucose-6-phosphate and mannose-6-phosphate, have shown wound healing and anti-inflammatory activity. Glucomannan and gibberellin stimulate fibroblast activity and proliferation, which turns to increase collagen content and thereby stimulating wound healing.

Some experimental studies conducted in diabetic animals have suggested that oral ingestion of *A. vera* may attenuate the diabetic foot wound. Daburkar et al[34] reported significant wound healing effect of *A. vera* gel extract in streptozotocin-induced diabetic rats. Oral administration of *A. vera* gel extract (300 mg/kg) either alone or in combination of topical application for a period of 9 days significantly improved glucose homeostasis, along with increased level of DNA and glycosaminoglycans (GAGs) in the granulation tissues. The results bring acceleration of ulcer healing and tensile strength.

Mechanism underlines wound healing activity of *A. vera* extracts may occur through improvement of insulin resistance and lowering blood glucose levels. Anti-inflammatory effects of *A. vera* could also be explained by an antioxidant mechanism since it attenuated free radical damage to the regenerating tissues.

GAGs, especially hyaluronic acid (HA), is believed to be one of the important factors to facilitate ulcer healing. Increased content of HA and other GAGs in the granulation tissue in response of *A. vera* administration is therefore supported that treatment potential of *A. vera* on DFU is, at least in part, occurred through its effect on GAGs.

Other proposed mechanisms related to healing effects of *A. vera* gel may include keeping wound moist, initiating epithelial cell migration, accelerating collagen deposition and maturation, and the reduction of inflammation.

While several studies demonstrated positive effects of *A. vera* gel to improve DFU, some others claimed it has no effect, or even delaying the healing process. Contradictory results found may be explained by the differences in the extraction process and stability of the active ingredients.

Naringin. Naringin is a major flavanone glycoside in grapefruit, which is responsible for the juice's bitter taste. Naringin is metabolized to the flavanone naringenin in humans. As a known antioxidant compound, it has also shown antidiabetic and anti-apoptotic properties. Healing potential of naringin in animal models of DFU was evaluated.[35] Daily administration of naringin (per os) at a dose of 40 or 80 mg/kg for 16 days to diabetic rats significantly reduced the wound area as well as modulating glucose homeostasis.

Naringin may facilitate wound healing through various mechanisms using such activities to promote angiogenesis, inhibiting endothelial apoptosis, reducing level of blood

Table 1. Summary of the Effect of Dietary Micronutrients and Natural Compounds Supplementation to Wound Healing in Diabetic Foot Ulcer.

Nutrient	Mechanism	Effect to Wound Healing	Reference
Vitamin D	Accelerated even if TGFβ1 levels are low	Reduced fibrosis in wound	9
Vitamin E	Revealed a reduction in plasma malondialdehyde level along with heightened levels of glutathione peroxidase activity	Speeded the closure of wound	13
Vitamin C	Promoted the levels of TGFβ1, MMP-1 expression and gelatinase activity while reduced TIMP-1 level	Repaired the monolayer through heightened collagen turnover	20
Vitamin A	Promoted epithelial cell differentiation while acting as a stimulant on the immune system	Improved macrophage activity and synthesis of collagen	7, 23
Magnesium	Interacted with ATP in the processing of collagen formation and involved in protein formation and tissue development	Reduced risk of developing diabetic foot ulcers	24-26
Zinc	Involved in the cross-linkage of collagen, DNA and RNA synthesis, protein synthesis, immune function, cellular proliferation	Accelerated wound healing and associated with venous leg ulcers and type 2 diabetic foot ulcers	7, 30
Copper	Involved in protein and collagen synthesis	Enhanced wound healing	7, 24
Iron	Required for hydroxylation in collagen synthesis	Reduced risk of developing diabetic foot ulcers	7, 31
Boron	Required for human nutrition and metabolism, but the mechanism is still unknown	Effects on diabetic wound healing has not been elucidated so far. Improved rates of wound repairing in STZ-induced diabetic rats	33
Aloe vera	Inhibited inflammation, stimulated fibroblast proliferation and increased collagen synthesis	Accelerated ulcer healing and tensile strength in STZ-induced diabetic rats	34
Naringin	Increased angiogenesis, inhibited cell death, reduced blood level of glucose and oxidative stress	Controlled glucose homeostasis and reduced wound area in diabetic rat model	35
Radix Astragali and Radix Rehmanniae	Inhibited inflammation, accelerated angiogenesis and tissue remodeling	Showed synergistic effects on increased rate of wound healing	36-38

Abbreviations: TGFβ1, transforming growth factor beta 1; MMP-1, matrix metalloproteinase-1; TIMP-1, tissue inhibitor of MMP-1; ATP, adenosine triphosphate; STZ, streptozotocin.

glucose, and oxidative stress. Molecular mechanisms underline its effects are proposed via upregulation of growth factors (IFG-1, TGFβ and VEGF-c) and downregulation of inflammatory cytokines (TNF-α, IL-1β and IL-6) expression. Thus, it is subsequently increased angiopoietin and collagen synthesis, which prevents delayed ulcer healing.

Radix Astragali and Radix Rehmanniae. Radix Astragali (RA) and Radix Rehmanniae (RR), are 2 principal components of a traditional Chinese medicine formula for treating diabetes and its complications, have been evaluated in their effectiveness in treating diabetic wounds.

Synergistic interaction between the extracts used as herbal formula has found efficiently enhanced rate of wound healing in the model of diabetic rats.[36] The combination stimulated proliferation of β-cell and secretion of insulin, thus simply ameliorate diabetic condition. Improvements of DFU healing occurred through various controlled processes of inflammation, angiogenesis, and tissue remodeling. RA played a prominent role in stimulation of fibroblast proliferation and inhibition of the inflammatory process. It had also been reported to moderate insulin resistant and meliorate endothelial dysfunction. RR, however, favored angiogenesis and tissue regeneration.[37]

Evaluation of the clinical effect of Chinese herbal medicine (CHM) treatment in terms of being effective and safe was publicized in a systematic review of randomized controlled trials (RCTs) of DFU.[38] RA, along with other herbal extracts are favored as active ingredients of herbal remedies for treating DFU that achieved positive effects in RCTs.

Meta-analysis of the data does favor the use of CHM to managing DFU. Ingestion of CHM combined with standard therapy tended to give better outcomes compared with using standard therapies alone. For example, a higher number of patients whose wounds were healed or showed significant reduction in wound area, and a larger mean of blood flow volume. On the contrary, few adverse events on the use of CHM had been reported, including dry mouth,

epigastric pain, nausea, and diarrhea. To address a definite conclusion, if herbal remedies were good enough for clinical practice, further high-quality RCTs are recommended.

Discussion

Chronic foot wounds are a common and challenging complication of diabetes, which causes higher rate amputation as well as prolonged hospitalization. Malnutrition causes improper metabolic alterations that affect the impaired wound healing process. Micronutrients, without the small quantities required, can cause serious adverse effects.

Understanding of the role of micronutrients and herbal remedies in the process of wound healing may bring out effective therapeutic approaches that resolve impaired diabetic wounds (Table 1). The use of micronutrients and natural compounds exhibit a wide range of health benefits to patients with diabetic foot ulcer. Zinc and various vitamins play a vital role in modulation of cell proliferation, collagen metabolism, and inflammation.

The role of antioxidant supplementation is apparently via inhibition of oxidative stress, which potentially diminishes infection and inflammation. Several plants have been demonstrated to have potential wound healing abilities, as well as their antidiabetic, antioxidant, and anti-inflammatory activity. Use of herbal remedy that combined various plant extracts could be more effective due to the synergistic effects of the various active compounds.

Oral supplementation of nutrients and/or herbal remedies can be effective; however, improper use of high-dose administration is considered harmful. Hence, further studies require, not only to demonstrate the effectiveness of the treatment but also to address its safety. Monitoring the uptake and body status of micronutrients should be integrated in routine clinical practice to conduct adequate supplementation which is safe and effective. Nevertheless, such additional aspects, including dosing, administrative route, timing of treatment, and role of individuals against combination therapy need to be clarified.

Nutrition is important for wound healing process in diabetic foot. Although there is less conclusive evidence due to small sizes in published RCTs, most researchers favor the role of nutritional supplements in promoting diabetic wound care.

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