*Journal of Pharmaceutical Research International*

***33(33B): 68-80, 2021; Article no.JPRI.69749***
***ISSN: 2456-9119***
*(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)*

# Therapeutic Role of Nitric Oxide in Diabetic Wound Healing: A Systematic Review

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors may use the following wordings for this section. Authors MMC and RVG managed literature review and wrote the first draft of the manuscript, authors YSO and GBC finalize the draft, managed the scope and suitabilility of review. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JPRI/2021/v33i33B31798
Editor(s):
(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Manoj Kumar Saurabh All India Institute of Medical Sciences, India.
(2) M. P. Kusuma, Osmania University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/69749

***Review Article***

***Received 20 April 2021***
***Accepted 24 June 2021***
***Published 29 June 2021***

## ABSTRACT

Post injury, healing of wound is essential for recovery of uprightness of the body, which is one of the complex, continuous and unanticipated chains of events in case of diabetic patients. Nitric oxide represents a potential wound therapeutic agent due to its ability to regulate inflammation and eradicate bacterial infections. Impaired wound healing is a prominent diabetic complication which may lead to amputations also. In addition to modern medicines we can use nitric oxide therapy prominently for diabetic wound healing. Prominent and proven role of nitric oxide as well as conventional materials (like metformin and hydrogen sulphide, whey proteins, acidified nitrile etc), therapies (like low level laser therapy, hyperbaric oxygen therapy etc) and techniques (like in vivo implants with biosensors) can be taken into consideration. Many plant extracts showed promising results for wound healing activity by increasing nitric oxide levels. Use of modern technologies such as implant with biosensor and technique like sonic head hog gene are available for diabetic wound healing using Nitric oxide. In this review, an attempt has been made to compile comprehensive updated information of role of nitric oxide in diabetic wound healing, which may be exploited by focusing more on development of effective strategies to treat diabetes-associated wound.

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*Keywords: Diabetes; L-arginine; nitric oxide; wound healing.*

## 1. INTRODUCTION

Nitric oxide (NO) is free radical produced from the amino acid L-arginine with the help of three distinct isoforms of nitric oxide synthase (NOS). Altered synthesis of NO has been correlated with pathophysiology of muscular dystrophies, nNOS is localised to the sarcolemma in mature muscle fibres so they interact with the dystrophin complex [1,2].(Table 1)

Nitric oxide regulates inflammation, chronic wound healing and diseases related bacterial infections. Mainly endogenous nitric oxide regulates systemic bacterial infection and exogenous nitric oxide gas is used as topical antibacterial agent [1,2].

Impaired wound healing is a prominent diabetic complication, where we can use NO therapy effectively [4]. Process of healthy wound recovery takes place within 30 days, whereas diabetic wound remains unresolved. Many researchers are focusing on developing effective strategies against diabetes-associated wound (a leading cause of amputations) [5].

### 1.1 Literature search strategy

The articles were searched from Flinders University library website. Databases like PubMed and ScienceDirect were used. Subheadings and keywords were identified from key concepts of the focus of interest of the study. The following keywords and subheadings were combined with AND or OR to proceed with the systematic literature (((nitric oxide* [Title/Abstract]) AND (wound healing* [Title/Abstract]))) AND (diabetes*[Title/Abstract]) Filters: Free full text, English, from 2005 – 2021 Articles were searched from all databases used in the search strategy. The titles and abstracts were screened and, duplicates and irrelevant articles were excluded according to inclusion and exclusion criteria. Full texts of eligible articles were retrieved, reviewed and a systematic review was constructed.

### 1.2 Historical Background

1970-Nitric Oxide known as noxious gas, found in cigarette smoke.

1977-Ferid Murad showed nitric oxide can increased tissue cyclic guanosine monophosphate (cGMP) levels and regulate enzymatic activity.

1987-Lgnarro and Salvador Moncada found that Nitric oxide control vital biological functions like vascular relaxation.

1992-Nitric Oxide was proclaimed as the “Molecule of the Year” [1,2,3,4].

## 2. ROLE OF NITRIC OXIDE IN DIABETIC WOUND HEALING

Normal wound causes haemostasis, inflammation, proliferation, and then tissue remodelling but in diabetic wound healing there is no normal healing timeline developed because diabetes mellitus is metabolic disorder and causes impaired wound healing [4].

Sometimes tissue damage is medium for bacterial growth and hypoxia condition in tissue is major cause of chronic wound. In 1990's, it was found that NO is involved in many physiological and pathological conditions. Nitric oxide plays very important role in many physiological and pathological conditions [3] (Fig. 1). Metformin, an oral antihyperglycemic agent, induce nitric oxide and inhibit lippopoly-saccharide (LPS) component of Cell wall of gram-negative bacteria which is activator of macrophage, which in turn is important for mammalian immune system and used in production of cytokines. Metformin is used for improvement of endothelial function in wound healing [6,7].

Prominent and proven role of nitric oxide as well as conventional materials (like metformin and hydrogen sulphide, whey proteins, acidified nitrile etc), therapies (like low level laser therapy, hyperbaric oxygen therapy etc) and techniques (like in vivo implants with biosensors) can be taken into consideration. Many plant extracts showed promising results for wound healing activity by increasing NO levels, using modern technologies like implant with biosensor and technique like sonic head hog gene can be used for diabetic wound healing.

### 2.1 Metformin and Hydrogen Sulphide

Metformin, an oral antihyperglycemic agent, increases endothelial NOS activity and activate the adenosine monophosphate and protein

kinase (AMPK) [8]. It helps to restore the blood flow in type-2 diabetes mellitus with the help of AMPK/eNOS mechanism. Metformin accelerate wound healing in mice that causes activation of ischemic muscle repair after 7 days of surgery [6,7]. Thrombospondin-1(TSP-1) is subunit of hydrogen sulphide ($H_2S$) and it is linked with three side of polypeptide (homotrimer). TSP-1 activates transforming growth factor-β (TGF-β) which is responsible for diabetic nephropathy.$H_2S$ used to regulate the inflammation by inhibiting the TGF-β Factor and high levels of plasma fibro gene found in type-2 diabetes mellitus is reduced by $H_2S$. Connective tissue grown from the wound increase Tumour Necrosis Factor alpha (TNF- α) in type-2 diabetes mellitus and in pro-inflammatory cytokines.$H_2S$ reduces the level of TNF-α protein expression and improves antioxidant and angiogenesis.$H_2S$ and NO are active in the endotheliocytes for maintaining vascular physiological function [9,10,11].Vascular endothelial growth factor (VEGF) releases NO and cGMP. $H_2S$ improves antioxidant and angiogenesis and reduce oxidative stress, which causes complications like diabetic nephropathy in the type-2 diabetes mellitus [9,10]. Heme oxygenase-1(HO-1) increased in diabetic animal treated with Streptozocin (STZ) and $H_2S$ [10]. HO-1 increase with decrease in iNOS and the restoration of vascular response caused by over expression HO-1 [12].

**Table 1. Properties of three NOS isoforms [3]**

| Property | Neuronal NOS (nNOS) | Inducible NOS (iNOS) | Endothelial NOS (eNOS) |
|---|---|---|---|
| Location | Brain, spinal chord | Macrophages | Endothelium |
| Major biological functions | Neuromediator, stroke, long term memory | Host defence, cytotoxic, inflammation | Vasodilator, Hypotension |
| Number of amino acids in predominant form | 1434 arachidonic acid | 1153 arachidonic acid | 1203 arachidonic acid |
| NO output | Low (p molar) | High (μ molar) | Low (p molar) |

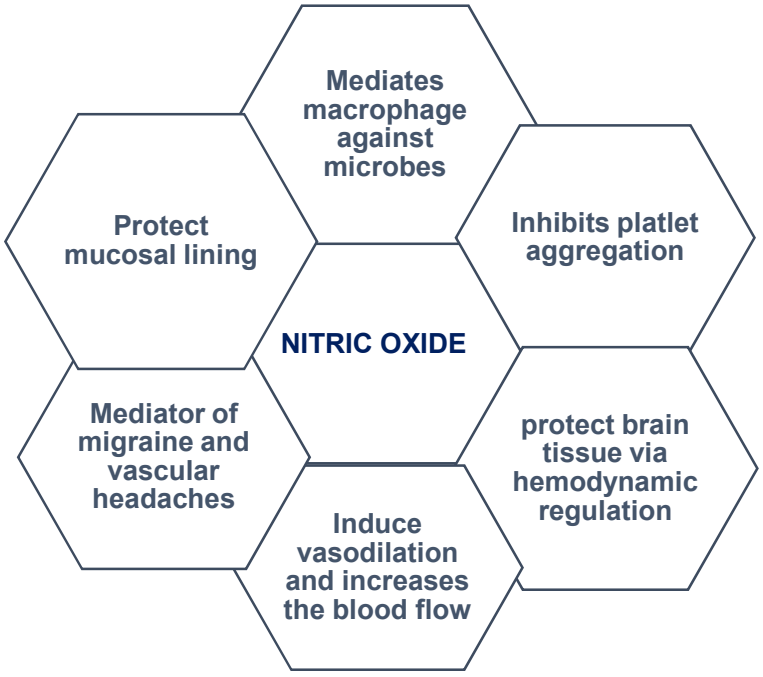


**Fig. 1. Role of nitric oxide [3]**

As $H_2S$ reduces production of malonodialdehyde dimutase activity (MDA) and activity of superoxde dismutane (SOD), which leads to HO-1 protein expression in wound [10]. MDA is responsible for decrease in GSH level in type-2 diabetus melitus. GSH in turn is responsible for increse in glutathione peroxidase and decrese in gluthion production in type-2 diabetus melitus. The effect of increase in $H_2S$ expression are summarized in Fig. 2 [9,10].

## 2.2 Traditional/Herbal Medicine

In traditional medicine, plant and natural source with antimicrobial, antioxidant, anti-inflammatory activity has been used for wound healing and reducing bacterial infection by reducing oxidative damage. The traditional medicinal plant extracts with nitric oxide are used for repairing of diabetes wounds [13,14,15].

*Helicteres isora* linn leaf extracts showed antioxidant and antimicrobial activity properties [13].*Brachylaena elliptica* and *Brachylaena ilicifolia* extracts were found effective against bacterial infection due to wound in diabetic patients [15]. *Salvia kronenburgii* Rech and *Salvia euphratica* Montbret showed *in vitro* antioxidant and antimicrobial activity against Staphylococcus aureus, *Escherichia coli* and Candida species [14].

NO-radical scavenging activity of medicinal plant extracts exhibit antioxidant properties due to presence of flavonoids, tannins, alkaloids and polypeptides. Flavonoids and saponins show antimicrobial activity and can be used against bacterial infection of diabetic wound [13,14,15].

The traditional Chinese medicine *Shixiang plaster* and Asiaticoside extracted from *Centella asiatica* show angiogenic antioxidant properties and are used to treat Diabetic cutaneous ulcer (DCU). Study of their effect on wound healing using Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) shows that both act by increasing vascular endothelial growth factor (VEGF), cluster of differentiation (CD34) and eNOS**.** In addition, *Shixiang plaster causes* reduction in expression *of* advanced glycosylation end product (AGEs) and suppresses vascular cell adhesion molecule-1(VCAM) & receptor for advanced glycation end products (RAGE) whereas Asiaticoside increases iNOS. AGEs and RAGE combine cause intracellular oxidative stress and activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which control transcription of DNA [16,17].

DCU wound regulation occurs by Wnt/β-catenin signalling pathway. Endogenous NO is insufficient to heal the wound associated with DCU so topical application of exogenous NO and Asiaticoside gel is recommended to promote wound healing along with traditional and modern medicine [17] (Fig. 3).

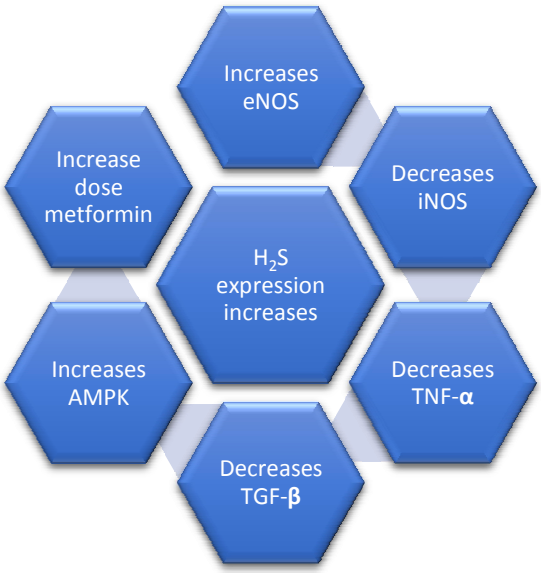


**Fig. 2. Effect of increse in $H_2S$ expression[10,11]**

Traditional medicine *Euphorbia* hirta linn contains flavonoids so its ethanolic extract is used orally or applied topically to prevent oxidative stress. Other traditional medicine like *Clinacanthus nutans* (act by NO scavenging) reduces oxidative stress and shows antioxidant and anti-inflammatory activity [18].

*Moringa oleifera* aqueous fraction is used in diabetic foot ulcer for wound healing. (Fig. 4)[19].

Topical application of Vicenin-2 (VCN-2), anti-inflammatory and antioxidant, is beneficial for diabetic wound healing. Experimental study of VCN-2 on laboratory rat shows decrease in proinflammatory cytokines TNF-α, COX-2, iNOS, NO via the nuclear factor kappa light chain enhancer of activated B cells (NF-kB) pathway. Results show significant decrease in blood glucose level in diabetic patient [20].

*Potentilla erectal* and *Potentilla genus* with antioxidant and antimicrobial potential showed wound healing property by promoting NO production. Promising results obtained in comparative study of wound healing in non-diabetic control (NDM), diabetic control (STZ-DM), and methanolic extract of *P. erecta*-treated (MEPE) in laboratory animals. (Fig. 5) [21].

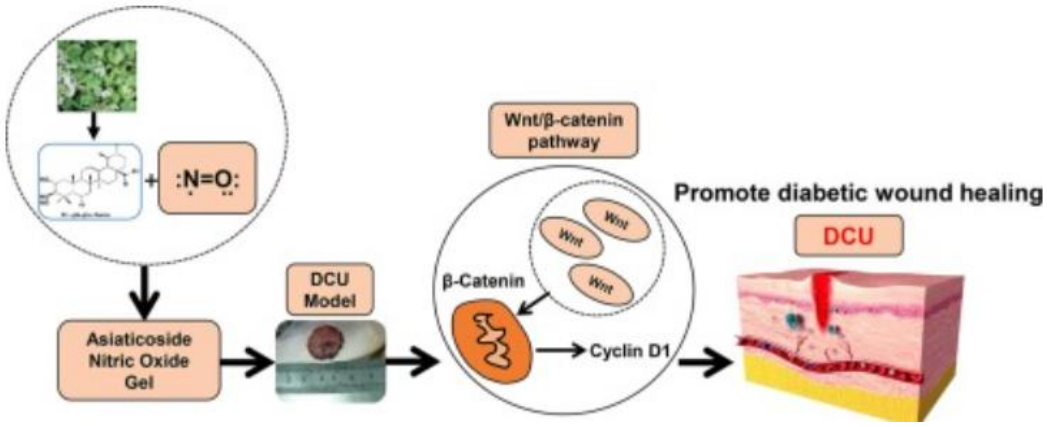


**Fig. 3. Asiaticoside nitric oxide promoted wound healing in DCU by Wnt/β-catenin pathway [17]**

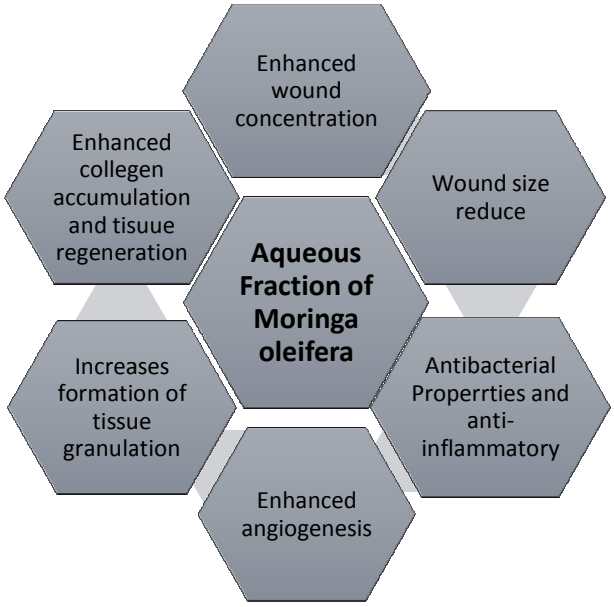


**Fig. 4. Role of *Moringa oleifera* aqueous fraction in diabetic wound healing [19]**

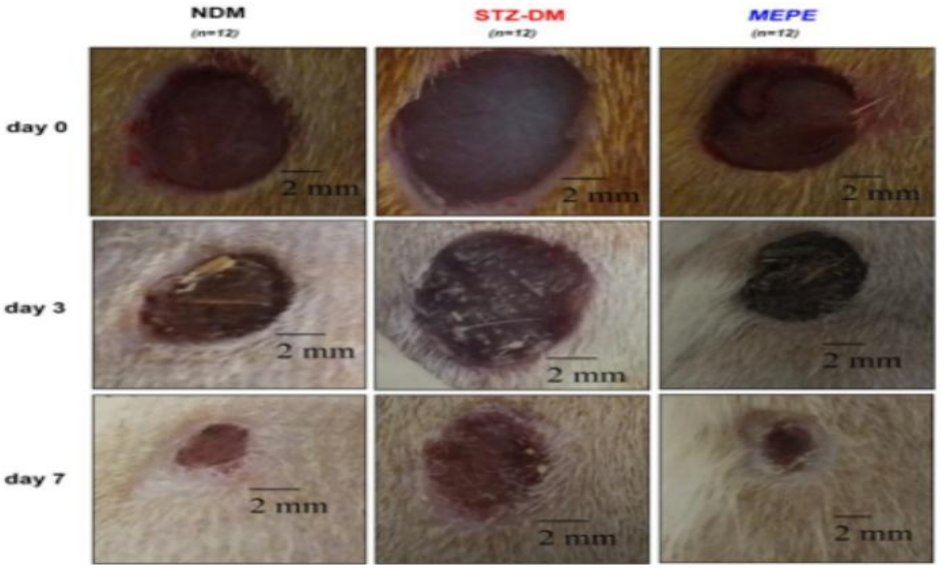


**Fig. 5. Wound healing in non-diabetic control (NDM), diabetic control (STZ-DM), and methanolic extract of *P. erecta*-treated (MEPE)[21]**

NO nanoparticle (NO-np) showed antimicrobial and wound healing properties against *Staphylococcus aureus* skin infection. *Staphylococcus aureus* is more infectious than Methicillin-resistant *Staphylococcus aureus* (MRSA) at soft tissue. Topically used powder of NO-np or np regulates wound healing (Fig. 6) [22].

*Byrsonima crassifolia* seed showed antidiabetics and wound healing properties. It is evaluated in STZ-induce type-1 diabetic rats and parameters were assayed like insulin level in pancreas, nitric oxide contents and oxidative stress [23].

## 2.3 Proteins

Type-2 diabetes causes endothelial cell dysfunction and glycosylation of extracellular matrix protein. Guanosine 5- triphosphate (GTP) is purine nucleoside, specific endothelial GTP cyclohydrolase-1 (GTPCH-1) over expression deal with unmanageable wound healing which causes limb amputation in diabetic patients. eNOS play important role in normal wound repair but is ineffective in STZ Induced type-1 diabetes because it reduces cofactor tetrahydrobiopterin (BH). BH cofactor of eNOS participates in oxidation of L-arginine and formation of NO [24]. Decreases level of BH result in production of $O_2$ instead of NO. Intracellular control by de novo synthesis pathway from GTP and rate limiting enzyme is GTPCH-1.(Fig. 7) [24].

Presence of constitutive NOS (cNOS) in type-1 diabetes suppress oxidative stress. Whey protein (WP) increases inflammatory action during cutaneous wound healing in rat. WP reduces radical oxygen and increases antioxidant glutathione. WP decreases excessive ROS, NO and malondialdehyde (MDA). Use of WP supplement enhances inflammation at initial stage of wound healing and decreases expression of IL-1β, TNF- α, IL-6, IL-4 and neutrophils in wounded diabetic (WD) experimental animals and later WP supplement restore these levels [25].

## 2.4 Therapy

Prolonged wound healing in diabetes comes with high social cost and challenges in clinical practice. Collagen synthesis by low-level laser therapy (LLLT) and hyperbaric oxygen therapy (HBO) are effective modalities for delayed wound healing [26,27].

LLLT used in treating diseases that increases oxidative stress like hyperglycaemia in diabetes, which causes increases ROS radical production. LLLT improves oxidative/nitrosative stress in the wound healing process in the experimental animals. It enhances the production of collagen and reduces oxidative stress, which suggests that use of LLLT may be possible remedy for treatement of diabetic wound [26].

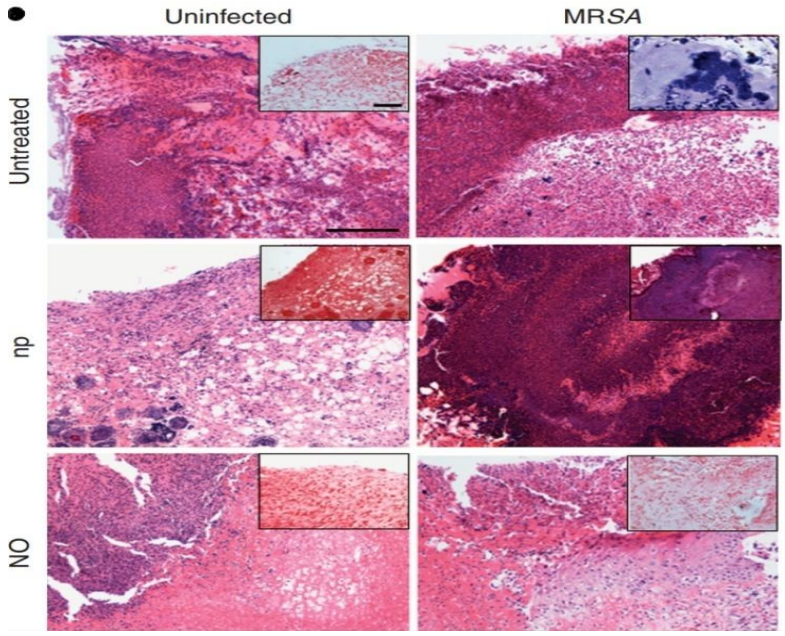


**Fig. 6. Effect of topical application NO-np or np on MRSAinfection [22]**

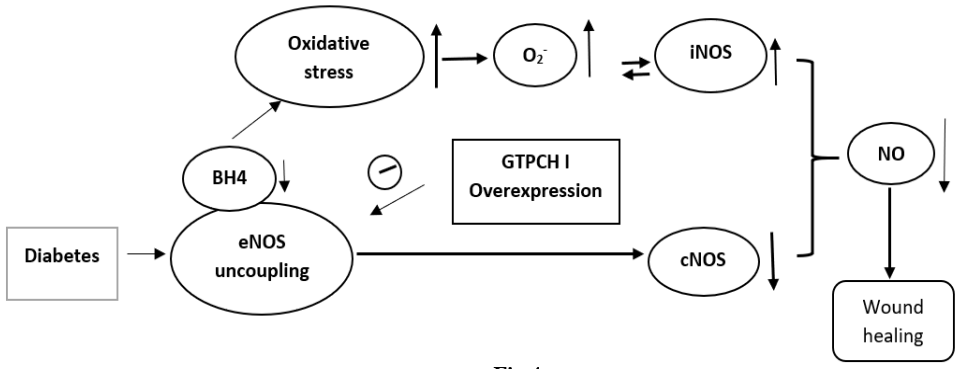


**Fig. 7. Wound healing in diabetes mellitus [24]**

HBO accelerate wound healing treatment in diabetic patient. Impaired wound healing take place because of patients having low circulating NO level due to absence of restoring action of insulin on NO synthesis. NO inhibitor causes increasing oxidative stress and HBO accelerate the effect of NO in wound healing. HBO help in the increasing TGF-β and LLLT help in increasing TNF-α and they promote formation of collagen. NO emerged as a critical mediator of tissue repair according to prominent ongoing clinical wound healing studies and experiments. After HBO short term treatment, the NO level significantly increased in wound fluid while remained constant in plasma [27].

## 2.5 Assay and Acetylation

Immunostochemistry assay and lysine acetylation improve wound healing. *In vivo* tissue oxyhaemoglobin ($HbO_2$) and oxygen saturation ($StO_2$) in visible wound in dermis causes increase in iNOS expression. Therefore, higher iNOS and reduction of $HbO_2$ and iNOS help in inflammation and prolong wound healing [28]. In Immunostochemistry expression of $HbO_2$ and $StO_2$ decreases [28].

Diabetic ulcer, skin repair, wound treated with sirtoin activator (enzyme which removes acetyl group) and class-1 Histone deacetylase inhibitor

(causes keratinoid cycle proliferation) improves wound healing via NO dependent mechanism [29].

NOS decreases the consumption of NO, which causes insufficient blood flow to tissue and responsible for pathogenesis and insensitivity in nervous tissue which results in prolong stimulation of diabetic skin ulcer.For diabetic skin ulcer effective analeptic strategies results in regulation of eNOS expression and increase levels of L-arginine [30].

Bioactive factor, cell and scaffolds are the element of tissue engineering which modifies statin loaded tissue engineering (TES) synthase by NO [30]. In *in vitro* experiment, TES statin increase NOS expression, high glucose induces TES and promote e NOS/ NOS synthesis for regeneration of tissue, which used in Human umbilical vein endothelial cell (HUVECs) [30].

In Gestational diabetes mellitus (GDM), hormones create by placenta stop the body from using insulin successfully and glucose present in blood get absorb in the cells. It was observed that in GDM there is increase in L-arginine transfer and hCAT-1 level whereas in reverse GDM there is increase in L-arginine uptake and human equilibrate nucleoside transporter-1 (hENT-1) level [30].

Statin causes increase in NO synthesis. There is increase in poly-ADP-ribose (PARP) level in diabetic and ischemic condition. PARP is highly active in diabetic condition that causes delay in wound healing & slow down the migration of HUVECs. Inhibiting PARP increases wound healing and promote angiogenesis in diabetic condition [31].

Fatty acid synthase (FAS) help in synthesis of palmitic acid which regulates de novo biosynthesis. De novo acetyl-CoA converts triglyceride in fat storage which maintains vascular repair and vascular injury balance through eNOS Palmitoylation. eNOS Palmitoylation reduce FAS in cell. Both FAS and eNOS decrease insulin deficiency and insulin resistant in diabetes (Fig. 8) [32].

## 2.6 Acidified Nitrile

Acidified nitrile improves wound healing in type-2 diabetes and increase dermis reconstruction of the cell collagen and deposition of tissue in experimental animals. VEGF level is measured in wound process. Acidified nitric oxide is effective in wound healing process. (Fig. 9)

Acidified nitrile in type-2 diabetes used to accelerates wound healing by quick rehabilitation of dermis and the enhancement neovascularisation and advance for collagen deposition in wound tissue [33].

Arginase play important role in L-arginine metabolism. Arginase slow down NO depletion during wound healing. iNOS in early phase of wound repair by inflammatory cells mainly in macrophages. After damage, NO release through iNOS regulates collagen formation. Factor involved in degradative pathway TGF-β and interleukin 4 (IL-4) decreases iNOS activity and increase arginase on other hand interferon-alpha (INF-α), IL-1 and lipopolysaccharide decreases Arginase and increases iNOS L-Hydroxy arginine and nitrile. it is intermediate of NO pathway nitrile and nitrate stable end product of NO pathway [1].

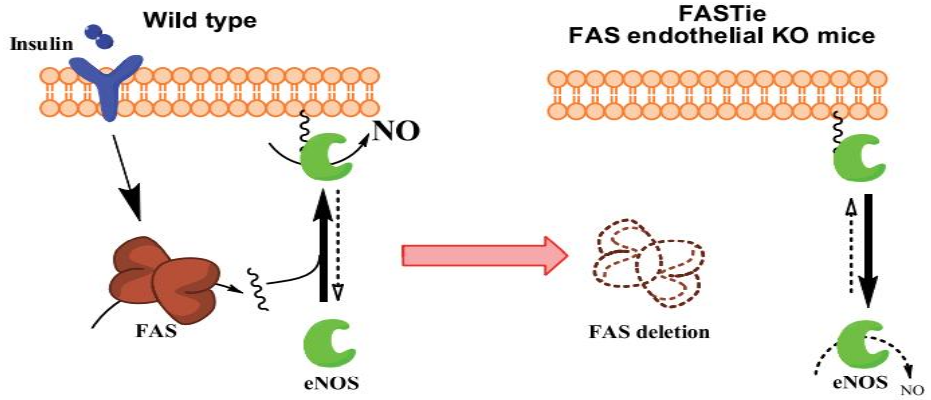


**Fig. 8. Decreased eNOS Palmitoylation in mouse model of diabetes [32]**

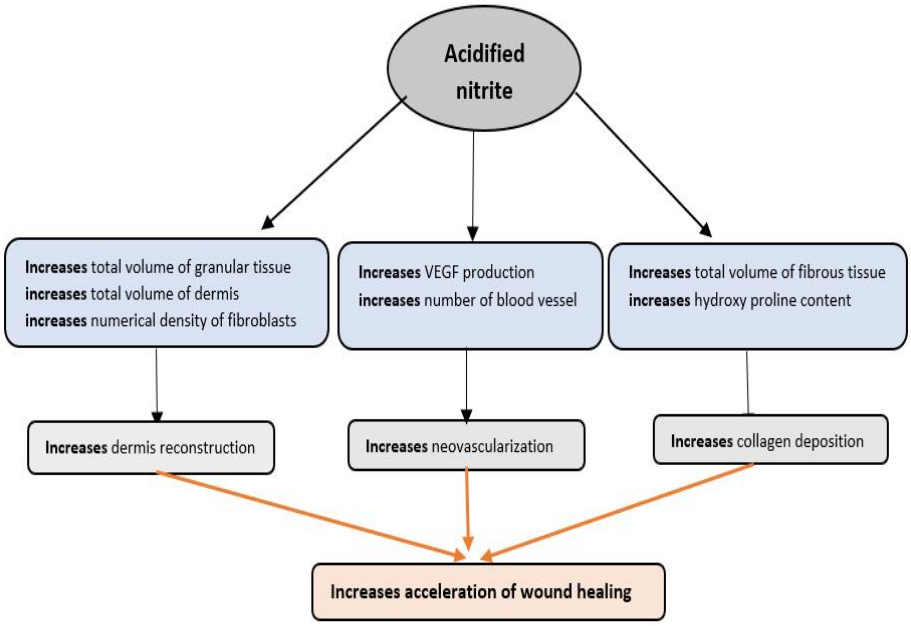


**Fig. 9. Process of diabetic wound healing by acidified nitrite [33]**

L-arginine and wound fluid NO and VEGF in the systemically L-arginine treatment take place in better wound healing the expression wound fluid more than topical L-arginine investigated oral captopril balance wound healing NO and VEGF expression in the wound fluid of diabetic rat [34].

### 2.7 Techniques Used in Wound Healing Using NO

Technique of *in vivo* implants of NO (with biosensor) is used for monitoring glucose level in patient. The insertion damage tissue and break proteins on surface of sensor which decreases glucose sensitivity. Biosensor site in macrophages recorded response of inflammation on sensors stress activity. Phagocytic activity causes *in vivo* sensor failure and polynuclear cell material affect oxidative damage. NO upregulate the VEGF and inflammatory cytokine involve in foreign body. For extended NO release, subcutaneous implant is capable to reduce inflammation and deposition of collagen reduces cytokinin production [35].

Sonic hedgehog gene (SHH), glycoprotein secreted by epithelial cell, involve in proliferation and embryonic patterning. It enhances release of cutaneous NO which is used to treat delay in wound healing in diabetes. SHH play an important role in postpartum tissue repair. SHH is signalling pathway of wound healing in cutaneous tissue and activate phosphatidylinositol 3-kinase (PI 3-kinase) pathway for activation of eNOS [36,37].

## 3. DISCUSSION ON RESULTS OF VARIOUS STUDIES

Prolonged wound healing in diabetes comes with high social cost and challenges in clinical practice therefore investigation for finding best remedy for that is aim of research studies. Few finding in situ are mentioned in this article. If we compare the findings by different scientists then we can recognize those for further studies. Metformin raises the hydrogen sulfide tissue concentration in body. Metformin is working to reclaim the effect on body to produce insulin naturally and hydrogen sulfide work to restore parent cell functions and activate the growth factor in type-2 diabetes [8,9]. Metformin act by increasing endothelial NOS activity and by activating the AMPK. Traditional plants with antimicrobial, antioxidant, anti-inflammatory activity able to reduce oxidative damage and can be used in diabetic wound healing. By increasing the activity of antioxidant glutathione WP is able to reduce the effect of oxygen radicals and lipid peroxidation and thus restore healing action [25]. LLLT help in increasing TNF-α whereas HBO help in the increasing TGF-β by promoting

**Table 2. Study of NO and wound healing [1].**

| Treatment | NO metabolites | Wound breaking strength | Collagen synthesis | Epithelialisation | Wound contraction |
|---|---|---|---|---|---|
| iNOS knock-out (excisional model) | Decreased | - | | Decreased | Decreased |
| iNOS knock-out (incisional model) | No effect | No effect | No effect | - | - |
| eNOS knock out | - | Decreased | | Decreased | Decreased |
| iNOS inhibition | Decreased | Decreased | Decreased | Decreased | Decreased |
| Arginine feeding | Increased | Increased | Increased | - | - |
| Arginine free diet | Decreased | | | | - |
| NO donor | Increased | Increased | Increased | | - |
| iNOS transfection | Increased | Increased | Increased | - | Increased |

formation of collagen and reduction of oxidative stress [26,27]. Techniques used in wound healing using NO are *in vivo* implants of NO (with biosensor) used for monitoring of glucose level in patient and SHH enhances release of cutaneous NO which is used to treat delay in wound healing and in postpartum tissue repair [35,36]. The effect of treatement by iNOS and Arginine on various parameters of wound healing are summarized in Table 2.

## 4. CONCLUSION

This review compiles the comprehensive updated information of role of nitric oxide in wound healing with detail underlying mechanisms. Use of metformin, hydrogen sulphide, traditional medicines or natural products used for diabetic wound healing with mechanisms and pathways involved. The current review reveals that metformin, $H_2S$ and antioxidants with NO expression could accelerated wound healing and stimulate angiogenesis. Metformin or $H_2S$ treatment in injury of muscle promotes wound healing in Type-2 diabetic patients. L-arginine is effective in systemic and topical wound healing in diabetic wound by increasing NO level but systemically more effective than topically.

The comprehensive information from the review will be helpful for researchers to focus on the preferential research areas yet to be examined and also to identify new techniques for effective diabetic wound healing.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENTS

The authors are thankful to the Dean and Management, School of Pharmacy, Dr. Vishwanath Karad MIT World Peace University, Pune-411038, India for their encouragement and for providing library facilities for preparation of this review.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Maria B, Witte MD, Adrian Barbul MD. Role of nitric oxide in wound repair. The American Journal of Surgery. 2002; 183:406-412.
2. Carman Ciervo DO, FACOFP, Christopher Zipp, DO MS. Nitric oxide in health and disease – its role in the practice of medicine. Osteopathic Family Physician. 2011;3:66-73.

3. Sahil Kumar, Rajesh K, Singh Bhardwaj TR. Therapeutic role of nitric oxide as emerging molecule. Biomedicine & Pharmacotherapy. 2016;85:182-201.
4. Maggie J, Malone-Povolny, Sara E. Maloney, Mark H. Schoenfisch. Nitric oxide therapy for diabetic wound healing. Advanced healthcare materials. 2019; 8(12):e1801210.
5. Que Bai, Kai Han, Kai Dong, Caiyun Zheng, Yanni Zhang, Qianfa Long, Tingli Lu. Potential Applications of Nanomaterials and Technology for Diabetic Wound Healing. International Journal of Nanomedicine. 2020;15:9713-9743.
6. Han X, Tao Y, Deng Y, Yu J, Sun Y, Jiang G. Metformin accelerates wound healing in type 2 diabetic db/db mice. Molecular medicine reports. 2017;16(6):8691–8698.
7. Yu JW, Deng YP, Han X, Ren GF, Cai J, Jiang GJ. Metformin improves the angiogenic functions of endothelial progenitor cells via activating AMPK/eNOS pathway in diabetic mice. Cardiovasc Diabetol. 2016;88:1-10.
8. Noriko Takahashi, MD, Rei Shibata, MD, PhD, Noriyuki Ouchi, MD, PhD,Masayuki Sugimoto, MD, PhD, ToyoakiMurohara, MD, PhD, and Kimihiro Komori, MD, PhD. Metformin stimulates ischemia-induced revascularization through an eNOS dependent pathway in the ischemic hindlimb mice model. Journal of vascular surgery. 2013;61:489-496.
9. Ciccone V, Genah S, Morbidelli L. endothelium as a source and target of h 2 s to improve its trophism and function. Antioxidant. 2021;10(3):486.
10. Guoguang Wang, Wei Li, Qingying Chen, Yuxin Jiang, Xiaohua Lu, Xue Zhao. Hydrogen sulfide accelerates wound healing in diabetic rats. International Journal of Clinical and Experimental Pathology. 2015;8(5):5097–5104.
11. Wang GG, Li W. Hydrogen sulfide improves vessel formation of the ischemic adductor muscle and wound healing in diabetic db/db mice. Iranian journal of basic medical sciences. 2019;22(10):1192-1197.
12. Ahmad M, Turkseven S, Mingone CJ, Gupte SA, Wolin MS, Abraham NG. Heme oxygenase-1 gene expression increases vascular relaxation and decreases inducible nitric oxide synthase in diabetic rats. Cellular and molecular biology. 2005;51(4):371-6.
13. Renuka Mahajan, Prakash itankar. Antioxidant, Antimicrobial and Wound Healing Potential of *Helicteresisora* Linn. Leaf Extracts.Digital Chinese Medicine. 2020;3:188-198.
14. SevdaGüzel, Yusuf Özay, MeltemKumaş, CoşarUzun, Ebru GökalpÖzkorkmaz, ZuhalYıldırım, MahmutÜlger, GizemGüler, Ayla Çelik, Yusuf Çamlıca, Ahmet Kahraman. Wound healing properties, antimicrobial and antioxidant activities of Salvia kronenburgiiRech. f. and Salvia euphraticaMontbret, Aucher&Rech. f. var. euphratica on excision and incision wound models in diabetic rats. Biomedicine & Pharmacotherapy. 2019;111:1260-1276.
15. Idowu Jonas Sagbo, Anthony Jide Afolayan, Graeme Bradley. Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria. Asian Pacific Journal of Tropical Biomedicine. 2017;7(9): 817–825.
16. Fei J, Ling YM, Zeng MJ, Zhang KW.Shixiang Plaster, a Traditional Chinese Medicine, Promotes Healing in a Rat Model of Diabetic Ulcer Through the receptor for Advanced Glycation End Products (RAGE)/Nuclear Factor kappa B (NF-κB) and Vascular Endothelial Growth Factor (VEGF)/Vascular Cell Adhesion Molecule-1 (VCAM-1)/Endothelial Nitric Oxide Synthase (eNOS) Signaling Pathways. Medical science monitor. 2019:25:9446-9457.
17. XuqiangNie, Han Zhang, Xiujun Shi, Jiufeng Zhao, Yu Chen, Faming Wu, Jianwen Yang, Xiaohui Li. Asiaticoside nitric oxide gel accelerates diabetic cutaneous ulcers healing by activating Wnt/β-catenin signaling pathway. International Immunoph armacology. 2019;79:1-15.
18. Tuhin RH, Begum MM, Rahman MS, Karim R, Begum T, Ahmed SU, Mostofa R, Hossain A, Abdel-Daim M, Begum R. Wound healing effect of Euphorbia hirtalinn. (Euphorbiaceae) in alloxan induced diabetic rats. BMC Complementary and alternative medicine. 2017;17(1):423
19. Muhammad AA, Arulselvan P, Cheah PS, Abas F, Fakurazi S. Evaluation of wound healing properties of bioactive aqueous fraction from Moringa oleifera Lam on experimentally induced diabetic animal model. Drug design, development and therapy. 2016;10:1715-1730.

20. Tan WS, Arulselvan P, Ng SF, Mat Taib CN, Sarian MN, Fakurazi S. Improvement of diabetic wound healing by topical application of Vicenin-2 hydrocolloid film on Sprague Dawley rats. BMC Complementary and alternative medicine. 2019;19(1):1-16.
21. Kaltalioglu K, Balabanli B, Coskun-Cevher S. Phenolic, Antioxidant, Antimicrobial, and *in-vivo* Wound Healing Properties of Potentilla erecta L. Root Extract in Diabetic Rats. Iranian journal of pharmaceutical research. 2019;19(4)264-274.
22. Luis R. Martinez, George Han, Manju Chacko, Mircea Radu Mihu, Marc Jacobson, Phil Gialanella, Adam J. Friedman,Joshua D. Nosanchuk and Joel M. Friedman. Antimicrobial and Healing Efficacy of Sustained Release Nitric Oxide Nanoparticles Against Staphylococcus Aureus Skin Infection. Journal of Investigative Dermatology. 2009; 129:2463–2469.
23. Gutierrez RM, Flores JM. effect of chronic administration of hexane extract of byrsonimacrassifolia seed on b-cell and pancreatic oxidative parameters in streptozotocin-induced diabetic rat. African Journal of Traditional, Complementary and Alternative Medicines. 2014;11(2):231-6.
24. Tie L, Li XJ, Wang X, Channon KM, Chen AF.Endothelium-specific GTP cyclohydrolase I overex pression accelerates refractory wound healing by suppressing oxidative stress in diabetes .American Journal of Physiology Endocrinology and metabolism. 2009;296 (6):1423-1429.
25. Ebaid H, Salem A, Sayed A, Metwalli A. Whey protein enhances normal inflammatory responses during cutaneous wound healing in diabetic rats. Lipids in health and disease. 2014;10:235:1-10.
26. Tatmatsu-Rocha JC, Ferraresi C, Hamblin MR, Damasceno Maia F, do Nascimento NR, Driusso P, Parizotto NA. Low-level laser therapy (904nm) can increase collagen and reduce oxidative and nitrosative stress in diabetic wounded mouse skin. journal of photochemistry and photobiology b: biology. 2016;164:96-102.
27. Gurdol F, Cimsit M, Oner-Iyidogan Y, Kocak H, Sengun S, Yalcinkaya-Demirsoz. Collagen synthesis, nitric oxide and asymmetric dimethylarginine in diabetic subjects undergoing hyperbaric oxygen therapy. Physiological research. 2009; 59(3):423-429.
28. Saidian M, Lakey JRT, Ponticorvo A, Rowland R, Baldado M, Williams J, Pronda M, Alexander M, Flores A, Shiri L, Zhang S, Choi B, Kohen R, Tromberg BJ, Durkin AJ. Characterisation of impaired wound healing in a preclinical model of induced diabetes using wide-field imaging and conventional immunohistochemistry assays. International Wound Journal. 2019;16(1):144-152
29. Spallotta F, Cencioni C, Straino S, Sbardella G, Castellano S, Capogrossi MC, Martelli F, Gaetano C. 2013. Enhancement of lysine acetylation accelerates wound repair. communicative and integrative biology. 2013;6(5):e25466.
30. Tamara Sáez, RocíoSalsoso, Andrea Leiva, Fernando Toledo, Paul de Vos,MarijkeFaas, Luis Sobrevia. Human umbilical vein endothelium-derived exosomes play a role in foetoplacental endothelial dysfunction in gestational diabetes mellitus. BBA- Molecular Basis of Disease. 2017;1864:499-508.
31. Xin Zhou, PhD, Darshan Patel, BS, Sabyasachi Sen, MD, Victoria Shanmugam, MD, Anton Sidawy, MD, MPH, Lopa Mishra, MD,and Bao-Ngoc Nguyen, MD. Poly-ADP-ribose polymerase inhibition enhances ischemic and diabetic wound healing by promoting angiogenesis. Journal of vascular surgery.2 016;65:1161-1169.
32. Xiaochao Wei, Jochen G. Schneider, Sherene M. Shenouda, Ada Lee, Dwight A. Towler, Manu V. Chakravarthy, Joseph A. Vita, and Clay F. Semenkovich. 2011.De Novo lipogenesis maintains vascular homeostasis through endothelial nitric-oxide Synthase (eNOS) Palmitoylation. The journal of biological chemistry. 2011;286:2933-2945.
33. Afzali H, Khaksari M, Jeddi S, Kashfi K, Abdollahifar MA, Ghasemi A. Acidified Nitrite Accelerates Wound Healing in Type 2 Diabetic Male Rats: A Histological and Stereological Evaluation. Molecules. 2021;26(7):1872
34. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, Carey Satterfield M, Smith SB, Spencer TE, Yin Y. Arginine metabolism and nutrition in growth. health and disease. National Institute of Health Journal. 2009;37(1):153-168.

35. Soto RJ, Merricks EP, Bellinger DA, Nichols TC, Schoenfisch MH. Influence of diabetes on the foreign body response to nitric oxide-releasing implants. Biomaterials. 2018;157:76-85.
36. Luo JD, Hu TP, Wang L, Chen MS, Liu SM, Chen AF. Sonic hedgehog improves delayed wound healing via enhancing cutaneous nitric oxide function in diabetes. American journal of physiology. 2009; 297(2):525-531.
37. Altaany Z, Moccia F, Munaron L, Mancardi D, Wang R. Hydrogen sulfide and endothelial dysfunction: relationship with nitric oxide. Current medical chemistry. 2014;21(32):3646-3661.



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