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Accelerating The Wound Healing Potential By Using Local Administration Of Insulin In Patients Presented With Diabetic Foot Ulcer

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Abstract

Background : Uncontrolled hyperglycemia carries a significant risk for delayed wound healing, which can cause lower extremity amputation and sometimes even cause death. A better alternative therapy is urgently needed due to the unfavourable adverse effects of the chemically prepared medications that are available right now. Insulin is abundantly used to manage hyperglycemia. Here we have evaluated the local insulin administration for the wound-healing potency of diabetic foot ulcers. Reduction in blood glucose level, increase in hydroxyproline in tissue homogenate of wound, and events like regenerating epithelial layer, vasodilation, angiogenesis, and epidermal degeneration proved the effectiveness of insulin administration. Likely, the superficial dermis shows proliferating dilated capillaries, and dense mixed inflammatory cell infiltrate composed predominantly of neutrophils and fibroblasts. Hence, based on the results of the present investigation, we could suggest that local insulin administration will be the better option for treating diabetic wounds.

Aim of the study : Accelerating the wound healing potential by using local administration of insulin in patients presented with Diabetic foot ulcer.

Methods :This prospective study was conducted at Government Cuddalore medical college, Dr MGR medical university, Tamilnadu, India, between January 2022 and April 2022, comprising 10 cases. Diabetic ulcer patients of both sexes are encouraged to include, but ages more than 70 yrs, immunodeficiency, pregnancy, under antibiotic therapy, pressure ulcer, post-operative surgical wounds, burns and other chronic wound cases are excluded from this study.To understand the wound healing process, the tissues samples of the wound was collected during the 0^{th} and 16^{th} day of treatment. Before collection of the tissue samples wound area was cleaned using sterile saline (0.9% NaCl) to eliminate the cellular debris. Aseptically, 2 mm of the tissue was dissected and placed in the 10% formalin solution. Further, all the samples were pre-examined for their visual appearance, and the nature of the samples was recorded. Results : Before and after the injection of insulin in the wound area, the blood glucose level was measured using a portable glucometer. The blood glucose level was presented in Fig 1, the ranges of 235.80±49.59 and 230 ±28.21mg/dl for the treatment and control group, respectively. No significant differences were found between the glucose level of the two groups ($p>0.05$); however, a slight variation in blood glucose level was observed after the injection of insulin. Before and after the injection of insulin, the qualitative assessment of wound is performed from day 0 to the end of treatment. A digital photograph of the wound was taken every day to monitor the progress of insulin application. A significant decrease in the wound area was noted in the insulin treatment group compared with the control one ($p<0.0001$). The results of wound contraction studies indicated the patients treated with insulin proceed with the

almost identical level of epithelialization. Considering non-treated samples, the collagen level was lesser or moderate compared to insulin treatment ($p<0.001$ after 4^{th} of treatment; student t-test). **Conculsion** :The results evidence that most of the tested plants showed positive responses to the process of wound healing. Ferulic acid accelerates the wound-healing process of diabetic-induced animals by decreasing the collagen, Zn and Cu levels and increasing serum antioxidant markers [28]. The diabetic rat had a higher range of wound contraction on 2nd day of treatment with Crude extract of Moringa oleifera, flowing the other parameter like TNF-α, IL-1β and IL-6 expression was significantly variated in comparison with control groups.

Keywords: Diabetic foot ulcer; insulin; local administration; histology

Introduction

The quantitative or qualitative variations of insulin resulted in hyperglycemia, a metabolic condition clinically termed Diabetics mellites [1]. Diabetes has appeared more often globally in recent years, and patients with diabetes have a more significant mortality and morbidity risk than the normal community [2]. As per the International Diabetes Federation projection that there were 415 million individuals with diabetes worldwide, with that number predicted to increase to 642 million by the year 2040 [3]. Diabetics mellites was associated with many life-threatening complications; the treatment options are relatively expensive. Because of elevated blood glucose, different complications occur of which Diabetic Foot Ulcer (DFU) is the most common and severe complication in diabetic populations. The ulceration takes place in the region between the knee and ankle. It is estimated that 22% of people with diabetes develop foot ulcers yearly, and their lifetime risk is around 34% [4, 5]. The following risk factors are the most common in DFU, including fluctuations in glycemic control, unhygienic and poor foot care, underlying peripheral vascular disease and complicated neuropathy [6]. These are all the major factors associated with osteomyelitis and amputation of lower extremities. Other than the physical complications, many factors are involved in creating open wounds during the life span of diabetic patients. Diabetes-related foot ulcers have a multifactorial aetiology. More than half of the patients with DFUs become infected, and more than 20% of mild to severe infections leads to amputations [7, 8]. Staphylococcus was found to be the most common etiological agent responsible for infection-related pressure ulcers.Diabetic-related ulcers can be treated by an appropriate therapeutic initiative that includes surgical debridement, off-loading of pressure, attention to infection by initiating specific antibiotic courses and vascular reconstruction-based on the necessity of the patients [9]. As a result of selection of appropriate therapeutic options can leads to averting lower extremity amputations [10]. However, even after successive healing of ulcers, recurrences have been observed. In the first three years following treatment, the recurrence rate was about 60%, and in the following five years, it was 65%. Besides the obvious immune dysfunction, diabetic patients also have slower collagen synthesis and accumulation, decreased angiogenesis, and poorer tensile strength of wounds, leading to higher rate of dehiscence [11]. On the other hand, a better alternative therapy is urgently needed due to the unfavourable adverse effects of the chemically prepared medications that are available now. Insulin is abundantly used to manage hyperglycemia. Some of the studies reported that insulin has wound healing activity in both human and animal models; however, no evidence was found about the safe use of insulin for diabetic wound management [12-16]. Insulin wet packing struggles to achieve the desired effect because of barriers such as the poor permeability of insulin, wound surface exudation, and tissue necrosis. Due to its ability to maintain a high concentration for a prolonged period and its efficacy, local insulin injection in the affected area seems more efficient [17]. Here we evaluated the local administration of insulin for the wound-healing potency of diabetic foot ulcers utilizing macroscopic, microscopic, and histological methods.

Materials and methods

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This prospective study was conducted at Government Cuddalore medical college, Dr MGR medical university, Tamilnadu, India, between January 2022 and April 2022, comprising 10 cases. Diabetic ulcer patients of both sexes are encouraged to include, but ages more than 70 yrs, immunodeficiency, pregnancy, under antibiotic therapy, pressure ulcer, post-operative surgical wounds, burns and other chronic wound cases are excluded from this study.

Treatment plan

The eschar and necrotic tissue attached to the wound were removed to enable proper administration of insulin. Meanwhile, the wound was regularly cleaned with a sterile saline solution to avoid the infection of specific microbes. The treatment was started by local administration of the intermediate insulin injection (Huminsulin 30/70; Eli Lilly and Company India Pvt Ltd) twice daily, 30-50IU for two weeks, as recommended by International Diabetic Federation and based on the blood glucose level.

Estimation of blood glucose level

The levels of blood glucose were monitored upon injection with insulin regularly starting from the 0 hours to 4 hours with 1-hour of time intervals to ensure the regulation of diabetics by using a digital glucometer (OneTouch Select Plus Simple Glucometer).

Macroscopic evaluation of the wounds

The wounds were evaluated over the study period with four days intervals (0, 4, 8, 12 and 16). For a better assessment of the wound-healing process, the scab of the wound was removed, and the area was measured. The percentage of wound contraction and epithelialization was calculated as follows

% Wound contraction = Size in 0^{th} day – size in nth day/Size in 0^{th} day * 100

% Epithelialization = Size of epithelialization 0^{th} day – size of epithelialization nth day/Size of epithelialization 0^{th} day * 100

Impact of insulin administration on hydroxyproline level

Measurement of hydroxyproline level was directly implicated in the level of collagen content. The tissue samples of the patient presented with diabetic foot ulcer were collected aseptically from 0^{th} day and 16^{th} day of the experimentation. Collected tissues were stored in the neutralized buffer and kept at -20℃ until further analysis. The concentration of hydroxyproline in the tissue homogenate was estimated per the previously described methods [18] and expressed as mg/100 mg dry tissue.

Histopathological examination

To understand the wound healing process, the tissues samples of the wound was collected during the 0^{th} and 16^{th} day of treatment. Before collection of the tissue samples wound area was cleaned using sterile saline (0.9% NaCl) to eliminate the cellular debris. Aseptically, 2 mm of the tissue was dissected and placed in the 10% formalin solution. Further, all the samples were pre-examined for their visual appearance, and the nature of the samples was recorded. Tissues were embedded in paraffin, cut at 5-µm sections, and stained with hematoxylin and eosin (H&E stain). Differential histological findings include epidermal regeneration, granulation tissue, inflammatory cells infiltration, angiogenesis, the proliferation of fibroblast cells and vasodilation.

Statistical investigation

The dataset generated during this study was processed using Microsoft excel. All the values were calculated and presented in terms of mean and standard deviation. Significant different among the groups were analysed using a t-test, 95% confidence interval was used in all the cases.

Variation in the blood glucose level

Before and after the injection of insulin in the wound area, the blood glucose level was measured using a portable glucometer. The blood glucose level was presented in Fig 1, the ranges of 235.80±49.59 and 230 ±28.21mg/dl for the treatment and control group, respectively. No significant differences were found between the glucose level of the two groups ($p>0.05$); however, a slight variation in blood glucose level was observed after the injection of insulin. Insulin injection in the wound area might have a positive impact on the reduction in the level of circulatory glucose level. There was a notable decrease in blood glucose level after 1hr administration, and it was retained for up to three hours (Fig 1). But it reached saturation peak at the 3rd hour of insulin injection ($p=0.057$). This behaviour evidenced that the local

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insulin administration has proven its efficiency towards reducing the blood glucose level.

Assessment of wound area and % of wound closure

Before and after the injection of insulin, the qualitative assessment of wound is performed from day 0 to the end of treatment. A digital photograph of the wound was taken every day to monitor the progress of insulin application. A significant decrease in the wound area was noted in the insulin treatment group compared with the control one ($p<0.0001$). The results of wound contraction studies indicated the patients treated with insulin proceed with the almost identical level of epithelialization. Further, to confirm the process of wound healing, the histo-microscopic observation was recorded. Throughout the study period, the time taken for epithelialization was prolonged in saline-dressed patients, whereas there was significant variation was observed in insulin-treated patients. On the other hand, the period of epithelialization also improved in response to insulin administration, as presented in Fig 2.

Collagen content

As shown in Fig 3, in the earlier stage of the healing process, the level of collage in the samples was comparably lesser in both groups, with the ranges of 1.11-5.34mg/of wound tissue. This might have happened because the wound healing process was yet to start; it was in the process of pre-inflammatory or inflammatory phase. Thus, minimising the collagen deposit around the wound area. During the treatment i.e samples collected between 4-12 days of treatment, a remarkable increase in collagen level was noted ($p<0.0001$; after 12 days of treatment). This demonstrated that the local administration of insulin has a positive sign on the wound healing process in the patient presented with a diabetic foot ulcer. Considering non-treated samples, the collagen level was lesser or moderate compared to insulin treatment ($p<0.001$ after 4^{th} of treatment; student t-test).

Histology

Microscopic observation of tissue sections will help us understand the wound-healing process. Fig 4 shows the H&E staining of day 0 and day 16 of diabetic wound samples. Tissue section from saline dressing showed that reduction in skin remodelling and poorly developed skin appendages like hair follicles and sebaceous gland; sections show skin with hyperkeratotic, acanthotic epidermis displaying areas of ulceration and regenerative atypia whereas it was significantly varied in insulin injected patients. Some sections from saline-dressed wound show skin with hyperkeratotic, irregularly acanthotic epidermis. The events like regenerating epithelial layer, Vasodilation, Angiogenesis, and Epidermal degeneration. Likely, the Superficial dermis shows proliferating dilated capillaries, and dense mixed inflammatory cell infiltrate composed predominantly of neutrophils and fibroblast.

Fig 1. Variations in the blood glucose level upon injection of insulin at wound sites

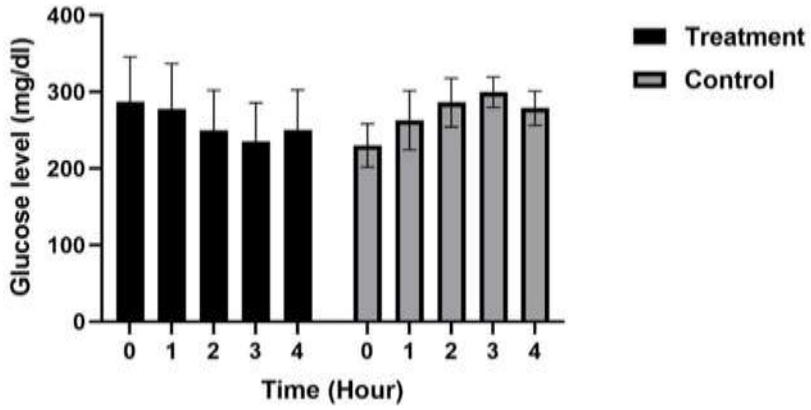

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Fig 2. Effects of insulin injection on wound contraction and wound closure. The data expressed in terms of mean and standard deviation. There was significant variation in the wound closure was noted between 0th day to other days of treatment ($p<0.0001$), except 4th day of treatment. Student t-test with Tukey's multiple comparisons test was adopted.

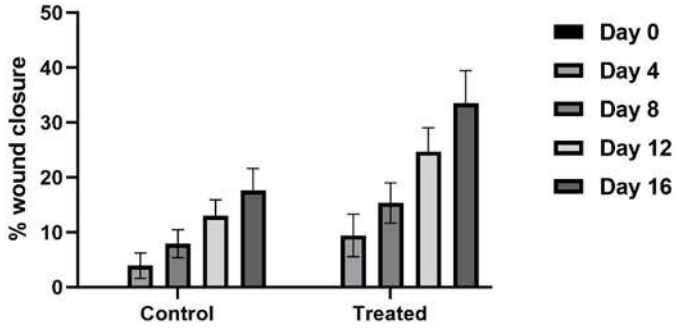

Fig 3. Effects of insulin injection on level of Hydroxyprolin level between the 0th day to 16th day of treatment upon insulin injection.

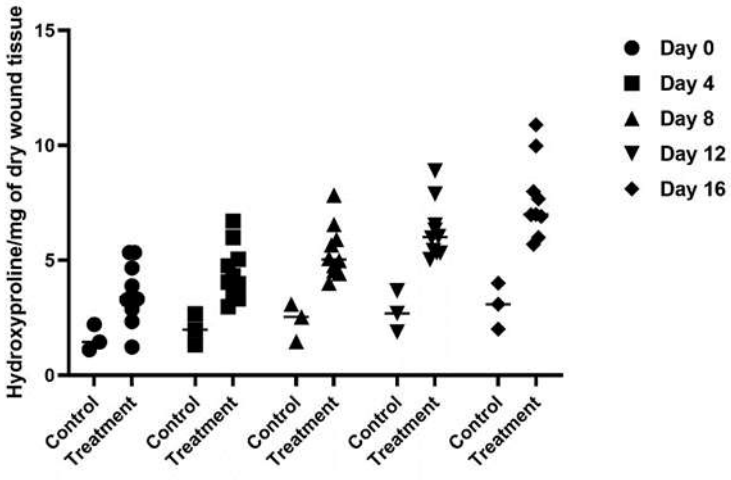

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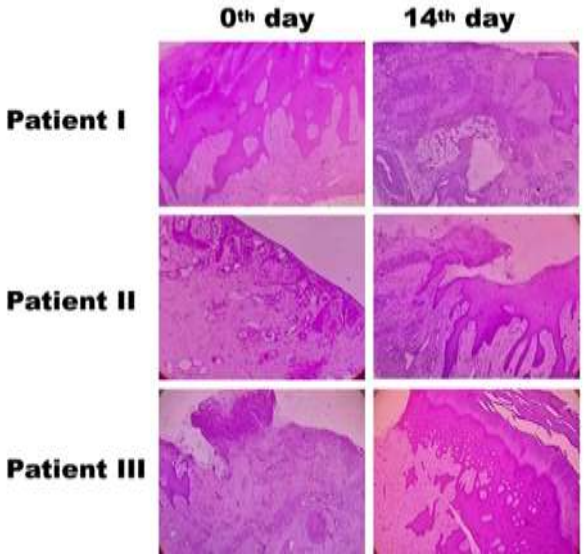
Fig 4. Histo-morphological variations tissues collected from the patients treated with injected with insulin. The tissue sections are stained with Hematoxylin and eosin stain. The data of the selected patients were presented below and compared 0th day and 14th day of treatment



Discussion

The incidence of diabetes has been sharply rising worldwide, attracting scientific and public interest. The patient's health, expenses, and quality of life are all significantly impacted. Uncontrolled hyperglycemia poses a significant risk for decreased wound healing, which, if untreated, can result in gangrene and amputation. Around ¾ of the patient who undergoes leg amputation have presented with type 2 diabetes. Insulin is recognised as one of the most important metabolic hormones, significantly involved in the regulation of blood glucose levels in human. Other than this, insulin possesses some other functions that include regulation of liver and muscular functions, Endothelium, and Vasculature and can increase glucose uptake in the spinal cord tissues and some brain regions [19]. It has a positive impact on wound healing, but the mode of administration is yet to be resolved. Likely, information on the safe use of insulin for diabetic wound management was unavailable. Here local administration of insulin has shown improvement in blood glucose management within 1-2hrs of injection as well it is a better choice rather than another type of dressing. No incidents like hypoglycemia, dizziness, palpitations and cold sweats were recorded in any patients. Topical administration of insulin reduces the size of the diabetic wound without making any adverse effects, like in the present study [20].

The occurrence of diabetic foot ulcers is a multifactorial process, which includes improver or poor management of blood glucose, infections, vascular disease, and neuropathy. Wound dressing is the major complication of wound management, thus directly affecting the quality of life. Also, it delays the process of healing and increases the hospital stay. Inflammation, cellular proliferation, and tissue regeneration are just a few illustrations of the stages that contribute complex, dynamic and regulated wound-healing processes. Zhang et al (2016) demonstrated the impact of systemic administration of insulin to heal the wound in diabetic patients. The study concluded that the insulin infection enhanced their healing process by strengthening the granulation and formation of new tissues; however, no significant difference was found between fasting blood glucose levels in both control and treated groups, respectively [17]. In this investigation, after injection with insulin, the level of blood glucose was dramatically dropped with reference to saline dressing groups. These observations are in accordance with previous reports [20-23]. Subcutaneous or intravenous administration of insulin is commonly recommended to manage hyperglycemia in diabetic patients. At the same time, this study has evidence that the reduction in blood glucose levels after local administration of insulin is like another mode of administration. However, the

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time duration of insulin action was significantly lesser than in other modes.

Wound contraction is a progressive mechanism to accelerate the healing process and generates enough granulation tissue to restore damaged tissue. One of the crucial steps in the healing process for wounds is re-epithelialization, which enables keratinocytes to move across the wound bed and regenerate epidermal layers. In the present study, a similar mechanism was observed in insulin-injected patients with an increase in epithelialization, thus directly responding to diabetic wound healing. Recently, nanotechnology facilitated the delivery of critical drugs, from antibiotics to chemotherapeutic agents, to specific sites to enhance bioavailability and biological activities. Nanofibrous scaffolds with glargine (insulin) infection at 3,7 and 14th day of treatment promoted diabetic wound repair through epithelialization [24]. Nanoparticles with human recombinant insulin promote wound remodelling by modulating inflammatory cytokines balance at the wound site [25]. In some cases, insulin promotes the healing of wounds in other parts of the body i.e skin donor site, Diabetic dry eye syndrome and corneal injuries, non-infected acute and chronic extremity wounds and grade 2 or 3 pressure ulcers [21, 23, 26, 27].

The major component of the extracellular matrix is collagen, and crucial phases in tissue repair and regeneration include collagen synthesis, deposition, remodelling, and maturation. We observed that the level of hydroxyproline was dramatically increased in diabetic wound tissues after the application of insulin, indicating the progress of wound healing through the accumulation of collagen in the surrounding tissues of the wound area. Further, histological studies confirmed insulin injection's effectiveness in healing diabetic wounds by presenting some of the hallmarks of tissue regeneration.

Traditionally, medicinal plants are evaluated for their wound-healing activity towards diabetic wound models. The results evidence that most of the tested plants showed positive responses to the process of wound healing. Ferulic acid accelerates the wound-healing process of diabetic-induced animals by decreasing the collagen, Zn and Cu levels and increasing serum antioxidant markers [28]. The diabetic rat had a higher range of wound contraction on 2nd day of treatment with Crude extract of Moringa oleifera, flowing the other parameter like TNF-α, IL-1β and IL-6 expression was significantly variated in comparison with control groups [29].

Conclusion

The results of this study indicate that local administration of insulin significantly accelerated wound contraction and re-epithelialization, increased collagen content, and improved wound healing in diabetic patients with uncontrolled blood glucose levels. The characteristic properties of insulin may contribute towards improved wound healing in hyperglycemic patients. As a result, the findings revealed that the injection of insulin to the local site dramatically speeds up wound healing of diabetic patients, suggesting that it may be used as a therapeutic for diabetic wounds.

Reference

1. Virally M, Blicklé JF, Girard J, Halimi S, Simon D, Guillausseau PJ. Type 2 diabetes mellitus: epidemiology, pathophysiology, unmet needs and therapeutical perspectives. *Diabetes Metab*, 2007; *33*(4), 231-244.
2. Bonow RO, Gheorghiade M. The diabetes epidemic: a national and global crisis. *The American journal of medicine* 2004; *116*(5), 2-10.
3. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, . Makaroff LE. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res. Clin. Pract*, 2017; *128*, 40-50.
4. Walsh JW, Hoffstad OJ, Sullivan MO, Margolis DJ. Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom. *Diabetic Medicine*, 2016; *33*(11), 1493-1498.
5. Sellman A, Katzman P, Andreasson S, Löndahl M. Presence of chronic diabetic foot ulcers is associated with more frequent and more advanced retinopathy. *Diabetic Medicine*, 2018; *35*(10), 1364-1370.
6. Pourkazemi A, Ghanbari A, Khojamli M, Balo H, Hemmati H, Jafaryparvar Z, Motamed B.

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Diabetic foot care: knowledge and practice. *BMC endocrine disorders*, 2020; *20*(1), 1-8.

7. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Senneville E. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin. Infect. Dis*, 2012; *54*(12), e132-e173.
8. Lavery LA, Armstrong DG, Wunderlich RP, Tredwell J, Boulton AJ. Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. *Diabetes care*, 2003; *26*(5), 1435-1438.
9. Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. *N. Engl. J. Med*, 2017; *376*(24), 2367-2375.
10. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, Mauricio D, Huijberts M. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. *Diabetologia*, 2008; *51*(5), 747-755.
11. Sung HJ, Johnson CE, Lessner SM, Magid R, Drury DN, Galis ZS. Matrix metalloproteinase 9 facilitates collagen remodeling and angiogenesis for vascular constructs. *Tissue engineering*, 2005; *11*(1-2), 267-276.
12. Lima MH, Caricilli AM, de Abreu LL, Araújo EP, Pelegrinelli FF, Thirone AC, Saad MJ. Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: a double-blind placebo-controlled clinical trial. *PloS one*, 2012; *7*(5), e36974.
13. Hrynyk M, Neufeld RJ. Insulin and wound healing. *Burns*, 2014; *40*(8), 1433-1446.
14. Abdelkader DH, Osman MA, Elgizaway SA, Faheem A, McCarron PA. The role of insulin in wound healing process: mechanism of action and pharmaceutical applications. *J. anal. pharm. res.* 2016; *2*(1).
15. Oryan A, Alemzadeh E. Effects of insulin on wound healing: a review of animal and human evidences. *Life Sciences*, 2017; *174*, 59-67.
16. Wang J, Xu J. Effects of topical insulin on wound healing: a review of animal and human evidences. *Diabetes Metab Syndr Obes*, 2020; *13*, 719.
17. Zhang Z, Lv L. Effect of local insulin injection on wound vascularization in patients with diabetic foot ulcer. *Exp. Ther. Med*, 2016; *11*(2), 397-402.
18. Ren J, Yang M, Xu F, Chen J, Ma S. Acceleration of wound healing activity with syringic acid in streptozotocin induced diabetic rats. *Life sciences*, 2019; *233*, 116728.
19. Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, Pang MG. Role of insulin in health and disease: an update. *Int. J. Mol. Sci.*, 2021; *22*(12), 6403.
20. Zagon IS, Klocek MS, Sassani JW, McLaughlin PJ. Use of topical insulin to normalize corneal epithelial healing in diabetes mellitus. *Archives of Ophthalmology*, 2007; *125*(8), 1082-1088.
21. Zhang XJ, Wu, X, Wolf SE, Hawkins HK, Chinkes DL, Wolfe RR. Local insulin-zinc injection accelerates skin donor site wound healing. *Int. j. surg. res*, 2007; *142*(1), 90-96.
22. Apikoglu-Rabus S, Izzettin FV, Turan P, Ercan FERİHA. Effect of topical insulin on cutaneous wound healing in rats with or without acute diabetes. *Clin. Exp. Dermatol.*, 2010; *35*(2), 180-185.
23. Cruz-Cazarim EL, Cazarim MS, Ogunjimi AT, Petrilli R, Rocha EM, Lopez RF. Prospective insulin-based ophthalmic delivery systems for the treatment of dry eye syndrome and corneal injuries. *Eur J Pharm Biopharm*, 2019; *140*, 1-10.
24. Lee CH, Hung KC, Hsieh MJ, Chang SH, Juang JH, Hsieh IC, Liu S J. Core-shell insulin-loaded nanofibrous scaffolds for repairing diabetic wounds. *Nanotechnol. Biol. Med*, 2020; *24*, 102123.
25. Kaur P, Sharma AK, Nag D, Das A, Datta S, Ganguli A, Choudhury D. Novel nano-insulin formulation modulates cytokine secretion and remodeling to accelerate diabetic wound healing. *Nanotechnol. Biol. Med*, 2019; *15*(1), 47-57.

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26. Rezvani O, Shabbak E, Aslani A, Bidar R, Jafari M, Safarnezhad S. A randomized, double-blind, placebo-controlled trial to determine the effects of topical insulin on wound healing. *Ostomy Wound Manage*. 2009;**55**(8):22–28.

27. Attia EA, Belal DM, El Samahy MH, El Hamamsy MH. A pilot trial using topical regular crystalline insulin vs. aqueous zinc solution for uncomplicated cutaneous wound healing: impact on quality of life. *Wound Repair Regener*. 2014;**22**(1):52–57.

28. Ghaisas MM, Kshirsagar SB, Sahane RS. Evaluation of wound healing activity of ferulic acid in diabetic rats. *International wound journal*, 2014; *11*(5), 523-532.

29. Azevedo ÍM, Araújo-Filho I, Teixeira MMA, Moreira MDFDC, Medeiros A.C. Wound healing of diabetic rats treated with Moringa oleifera extract. *Acta cirúrgica brasileira*, 2018; *33*, 799-805.

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