Introduction

Every 30 seconds, one lower limb amputation due to diabetes is performed in the world (1). Diabetes is the main reason for non-traumatic lower-limb amputations. Diabetic foot ulcers (DFUs) are common complications of patients with uncontrolled diabetes (2). The mortality rate of DFUs is ~50% after five years (3). DFUs are a chronic, non-healing, tricky situation of diabetes leading to high hospital charges, and in severe cases, to amputation. Circumferential vascular ailment, neuropathy, atypical cellular, and cytokine commotion are the core elements that obstruct diabetic wound healing (4).

Leech therapy has been used for many types of diseases since ancient times (5). Medicinal leech (Hirudo orientalis) was used by Avicenna, the great Iranian philosopher and physician (980-1037 A.D.), for the treatment of different diseases. The morphology of the leech was described by Avicenna in his book Canon of Medicine (6). The same morphology was described by Utevsky and Trontelj in 2005 and named Hirudo orientalis. The therapeutic effects of the leech are due to the presence of proteins in their saliva, which enter the body when they suck the patient’s blood (7). Using two-dimensional electrophoresis, >100 specific proteins have been obtained from the leech salivary gland secretion, and the effects of eight proteins from the medicinal leech have been confirmed. Unfortunately, the use of leech therapy has been restricted because of the possibility of infection by the microbial flora of the leech’s digestive tract. The prevalence rate of 4.1% for the side effects of using leeches was reported by Mercer et al. (1987) and later, similar articles reported infection up to 36.2% (Bauters et al., 2007) (8). Based on this, to prevent possible complications, we decided to provide local medicinal products from leeches.

Case Report

Every 30 seconds, one lower limb amputation due to diabetes is performed in the world. This study was conducted on a 64-year-old woman with a diabetic foot on her left leg. The patient had pain with a visual analog scale (VAS) score of 80 mm on 100 mm. This study aimed to evaluate the effect of a product derived from leeches in the treatment of DFUs. Wound grade 1 according to Wagner’s classification system was covered with leech cream. A decoction of Hirudo orientalis was prepared by lyophilizing leeches at ~80°C, the proteins containing biologically active substances (BAS) were extracted with normal saline, and this BSA was formulated in the form of cream. In vitro control of this pharmaceutical product was estimated according to the activity of these BSA. The leech cream was applied twice a day from the knee to the tail of the left toe for a month. The patient used no antibiotics and only received hygiene. After treatment, the patient was followed up for two months. Pain decreased significantly in terms of VAS: 40 mm after two days. At the end of the 3rd day, the pain completely disappeared. The granulation tissue was apparent on the toes’ ulcers after a week. The diameter of the wound was reduced by ~50% in three weeks. After four weeks, the ulcers were completely healed. After two months follow up there was no sign of wound recurrence. Topical leech cream enhanced the wound healing process and, therefore, may have curative effects on DFUs.

Keywords: Hirudo therapy, Leech therapy, Diabetic foot ulcer, Gangrene, Case report
Chief Complaint and Presenting Illness

A 64-year-old homemaker woman presented with a history of uncontrolled type 2 diabetes (A1C>7%) and painful foot wounds on the left leg for a year on 17 December 2019. During daily debridement, there was no bleeding in the wound that was washed with physiological serum and dressed with fibrinolysin and phenytoin creams. In terms of Wagner’s classification system, the DFU was of grade 1 (superficial ulcer of the skin or subcutaneous tissue) (9). The left first toe and the middle toe were involved, and the size of the wound was 3.5×2 cm. The patient had pain with a visual analog scale (VAS) score of 80 mm on 100 mm (10).

The patient's objective findings were tenderness and coldness around the wound, hairless limb, decreased dorsalis pedis arterial pulse, and tactile sensation relative to the right foot. There was no vascular claudication.

As for the past medical history, she had uncontrolled type 2 diabetes for the past year, and hypertension for two years ago. Moreover, she had a heart attack and a cerebrovascular accident several years ago.

She took losartan 50 mg twice daily, Glucophage 500 mg twice daily, Nitrocontin 2.6 mg twice daily, Gabapentin 300 mg every night after dinner, Praglinide 1 mg twice before meals, Concor 5 mg twice daily, atorvastatin 20 mg/day, Lanoxin 0.05 mg/day except for Fridays and Mondays, acetylsalicylic acid (ASA) 80 mg/day, Nephrovit daily, and amlodipine 5 mg/day. These drugs were continued during the patient's wound-specific treatment.

On physical examination, the patient was alert, with BP: 140/90 mmHg, heart rate: 105/min, respiratory rate: 16/min, and venous filling time: 10 seconds.

Laboratory tests were as follows: fasting blood sugar: 350 mg/dL. (normal range: 70-110 mg/dL), 2-hour postprandial blood sugar: 370 mg/dL. (normal range: <145 mg/dL), Hb A1C: 6.9 g/dL (normal range: <5.7%), albumin factor 1.9 (normal range: up to 4), hemoglobin (Hb): 9.60 g/dl (normal range: 12.3-15.3 g/dL), hematocrit (Hct): 30.80% (normal range: 35.9-44.5%), mean corpuscular volume (MCV): 90.30 FL (normal range: 77-96 FL), white blood cells (WBC): 4.60 m/mL (normal range: 4.4-11.3 m/mL), neutrophils: 54%, lymphocytes: 44%, eosinophils: 1%, red blood cells (RBC): 3.41 m/mL (normal range: 4.1-5.1 mL), platelets: 193 m/mL (normal range: 150-450 m/mL), blood sugar (PP): 400 mg%, serum bilirubin: 0.6 mg/dL, serum glutamic oxaloacetic transaminase (SGOT): 17 IU/L, serum glutamic-pyruvic transaminase (SGPT): 29 IU/L, serum alkaline phosphatase: 226 IU/l, total cholesterol: 148 mg/dl, serum triglyceride: 233 mg/dl, blood urea: 35 mg/dl, serum creatinine: 1.32 mg/dl, serum uric acid: 5.2 mg/dl, bleeding time: 1:20 minutes, and clotting time: 5:20 minutes.

The patient was treated with 5% leech cream prepared using lyophilized leech at −80°C and formulated in the pharmaceutical form of oil/water. In step one, wounds were washed and debrided without bleeding (Figure 1).

The cream was applied twice a day for a month from the knee to the tail of the toes. Every three or four days, and on the follow-up visit, a photo was taken of the wound (before applying the cream). A week after the application of the cream, bleeding was observed during the dress changing. The diameter of the wound was reduced and, after a month, the wound was completely healed, as displayed in Figure 1.

After applying the cream for a week, bleeding occurred when changing the dressing of the wound, which clearly indicated the improvement of the venous blood supply; this was not observed with other parallel treatments. Another important feature of this treatment compared to other methods of treatment is the trypsin inhibitor enzyme, inhibiting the trypsin and helping protect the wound and accelerate the healing process. Also, pain severity was significantly reduced (VAS: 4) after two days. At the end of the 3rd day, the pain was significantly reduced (VAS: 0).

Figure 1. Diabetic foot ulcer a) baseline; b) after 2 weeks of treatment; c) after 4 weeks of treatment and d) follow-up four weeks after cessation of treatment.
completely disappeared. New tissue was found in the wound after a week. The wound size was reduced by ~50% during the 3rd week. The diameter of the wound was decreased and, after one month, the wound was completely healed. Then, after four weeks, the ulcers were completely healed (Figure 1c). The follow up was done for two months and no recurrence was observed (Figure 1d).

**Table 1. Laboratory tests**

| Test items      | Value     | Normal range          |
|-----------------|-----------|-----------------------|
| WBC             | 4.6×10³/µL| 3.9-11.1×10³/µL       |
| RBC             | 3.41×10⁶/µL| 4.5-2×10⁶/µL         |
| Hb              | 9.60 g/dL | 12-16 g/dL           |
| HCT             | 30.80     | 33-48%                |
| PLT             | 172 m/ml  | 150-450 m/ml         |
| FBS             | 146 mg/dL | 70-110 mg/dL         |
| 2hpp            | 370 mg/dL | < 145 mg/dL          |
| HB A1c          | 6.9 g/dL  | < 5.7%                |
| ATrogen Factor  | 1.36      | Up to 4              |
| Triglyceride    | 72 mg/dL  | Desirable: < 150 mg/dL, Borderline: 150-199 mg/dL, High: 200 mg/dL |
| Cholesterol     | 131 mg/dL | Desirable: < 200 mg/dL, Borderline risk: 200-239 mg/dL, High risk: > 239 mg/dL |
| HDL             | 33 mg/dL  | Low Risk: > 60 mg/dL, Normal Risk: 40-60 mg/dL, High Risk: < 40 mg/dL |
| LDL             | 45 mg/dL  | Desirable Risk: <130 mg/dL, Medium Risk: 130-159 mg/dL, High Risk: >159 |
| LDL/HDL         | 1.36      | Desirable: < 5.4, Borderline: 5.4-7, High Risk: 7-11 |
| Uric acid       | 4.9 mg/dL | 3-7 mg/dL            |
| BUN             | 46 mg/dL  | 15-40 mg/dL          |
| Cr              | 1.32 mg/dL| 0.7-1.4 mg/dL        |
| SGOT            | 20 U/L    | 8-31 U/L             |
| SGPT            | 22 U/L    | 7-35 U/L             |
| Calcium         | 9.4 mg/dL | 8.6-10.6 mg/dL       |
| T4              | 12.1 ug/dL| 4.5-12.6 ug/dL       |
| TSH             | 1.82 mIU/L| 0.32-5.2 mIU/L       |
| 25OH Vit D3     | 58.6 ng/mL| Deficient<10 ng/mL   |

**Discussion**

The wound-healing effect of this product is visible in this study. This treatment seems more rational compared to the previous treatments of DFUs which commonly include debridement. Improving blood flow is a therapeutic principle in DFUs. In a study conducted on 564 diabetic patients with limb-threatening ischemia, PTA (percutaneous transluminal angioplasty) or BPG (bypass grafting) reduced member amputation. This feature is powered by hirudin and other anticoagulant agents present in the leech saliva. The salivary gland secretion of leeches contains proteins and peptides with different pharmacological activities. The mechanism of action of only eight known proteins from the leech salivary gland secretion that are biologically active includes: saratin protein with 12 kDa (molecular weight) and the mechanism of platelet adhesion to the damaged vascular wall, calin protein with 65 kDa, desubtilase-lysozyme with 12.6 kDa and the mechanism of antibacterial activity, hyaluronidase with 28.5 kDa and the mechanism of smiting hyaluronic acid in the cellular matrix (8), collagenase with 100 kDa and the mechanism of platelet initiation, antistasin with 15 kDa and inhibition of tissue damage or inflammation, and hirudin with 8 kDa and the mechanism of anticoagulation activity (11).
Early diagnosis and effective treatment are considered essential for wound healing because they can prevent amputation and preserve the quality of life of the patient. Therefore, specific treatments should be considered based on the size, depth, and location of the wound (12). Basic and standard principles for DFUs include nutrition and control of blood sugar, infectious control, proper debridement, vascular insufficiency and decreased blood flow, foot pressure, and moisture retention (13, 14). In recent decades, leech therapy has attracted surgeons’ attention for organ transplants and is used to prevent rejection. Because of the possibility of infection by the microbial flora of the digestive tract of leeches, despite the positive effects of leech therapy, their use has been associated with risks. Reports on the side effect of leeching indicates the incidence to range from 4.1% to 36.2%. Ischemia, neuropathy, and infection are three important pathological factors leading to complications of the diabetic foot. Although not all wounds can be prevented, they can be reduced (12). Sig et al. showed that leech enzymatic activity reduced infection due to its anti-inflammatory and antimicrobial effects, and also reduced ischemia due to anticoagulant effects, blood flow increase and regulation, and platelet function inhibition. Also, they mitigated wound infection and accelerated the state of wound healing (15).

**Conclusion**

This study demonstrated that the daily application of topical leech cream can effectively enhance wound healing in diabetic wounds in a mean period of four weeks without any adverse side effects while also contributing to patient outcome. Several treatments are employed for DFUs, and their goal is to preserve the organ. In previous treatments, the probability of treatment failure was high and treatment often led to amputation. These treatments only include the debridement and disinfection of necrotic tissue. In this study, the main goal was to improve blood supply and prevent tissue damage; with improved blood supply, tissue repair can also be achieved. Further investigation is required with more clinical trials. It should be notes that treatment should be tailored to each patient’s condition, health policy, and economic status.

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**Conflict of Interest**

Authors declared no conflict of interests.

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