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Analysis of VEGF AND TGFB1 proteins in normal and streptozotocin-induced diabetic rats, during treatment of formulations of *Aloe Vera*, *Henna*, *Adiantum capillus-veneris*, and *Myrrha*

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ABSTRACT

Background and objectives: Among the most important factors in wound healing pathways are transforming growth factor beta1 (TGFβ1) and vascular endothelial growth factor (VEGF). Botanicals are traditionally used for healing of different types of wounds. In this study, one mixture of plant materials such as *Adiantum capillus-veneris*, *Commiphora molmol*, *Aloe Vera*, and *Henna* were used to treatment of wound in diabetic and non-diabetic rats.
Methods: The dried leaves and resins were crumbled into a powder and mixed in equal parts with Vaseline. This mixture has been used as an ointment on the induced wounds in 60 diabetic and non-diabetic rats. Localization of TGFb1 and VEGF proteins as important healing markers were evaluated by immunohistochemistry and the level of TGFβ1 and VEGF proteins were detected by Western blotting.
Results: Immunohistochemical and Western blot analysis revealed that, VEGF in diabetic rats that treated with herbal mixture were significantly ($p < 0.05$) increased as compared with diabetic rats that treated with Vaseline at 7, 14, and 21 days after treatment. TGFβ1 in diabetic rats, treated with herbal mixture compared with diabetic rats treated via Vaseline showed no significant ($p > 0.05$) difference at 7, 14 and 21 days post wounding. TGFβ1 was significantly different ($p < 0.05$) between diabetic and non-diabetic rats treated with the herbal mixture at 7, 14 and 21 days after treatment.
Conclusions: The present study demonstrated that the mentioned herbal extracts might be effective in wound healing through the improvement in the amont of TGFβ1and *VEGF-A* proteins in wound site of diabetic rats.

1. Introduction

Diabetes mellitus is a chronic metabolic syndrome described through hyperglycemia and conflicts in carbohydrate, fat and protein metabolism [1,2]. In diabetic patients significant delay in development of granulation tissue and impaired cutaneous wound healing [3]. Foot ulcers, a severe damage in diabetic patients, is related with both mutilation and death [1,4,5]. Thus, the research seems necessary to expedite these matters.

Transforming growth factor beta (TGF β1) is a multifunctional cytokine, released from fibroblasts and performe on these cells [6]. Non-healing wounds almost show a loss of TGFβ1 signalling [7,8]. Vascular endothelial growth factor (VEGF) is a multifunctional growth factor produced by fibroblasts, keratinocytes, myocytes, endothelial cells and macrophages [9]. VEGF-A, previously known as VEGF, efficiently accelerate the proliferation and migration of endothelial cells *in vitro* [10]. Studies on wound healing development in diabetic animals have revealed that, VEGF and the amount of granulation tissue are intensely reduced [11,12].

The use of herbal based drugs to treatment of different types of wounds in many countries has a long history [7,13]. *Aloe Vera* (*Liliaceae*) with known antifungal, antimicrobial, anti-diabetic, and hypoglycemic properties has been used in traditional medicinal as a cathartic or remedy of burns and wounds [14,15]. Morgan et al.

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reported that *Aloe Vera* would initiate angiogenesis and wound repair by up-regulation of the VEGF and the TGFβ1 gene expression [16]. Studies have indicated that the use of oral or topical *Aloe Vera* improves wound healing by affecting the different phases of inflammation, collagen synthesis, maturation and wound closure in diabetic rats [17–19].

Commiphora molmol (Myrrha) produces resin with antibacterial, antifungal and antidiabetic properties [20,21] and is used currently for wound curation, intestinal disorders, diarrhea, coughs, and inflammation [22,23]. *C. molmol* contains many substances such as terpenoids, steroids, flavonoids, sugars, and lignums [24]. Lotfy et al. reported that the combined use of honey, bee propolis, and Myrrha promoted healing of wounds in a patient with diabetes mellitus [25]. *Adiantum capillus-veneris* has a long history in the traditional medicine with anti-inflammatory, antidiabetic, anti-infective, antimicrobial, and antioxidant properties [26]. *A. capillus* has significant angiogenic capacity of wound healing *in vitro* [27]. These properties suggest that local administration of *A. capillus* could have healing capability. *Henna* (*Lawsonia inermis*) is a well-known plant that is widely used to treat headaches, boils, and skin inflammation. Experimental and clinical studies have demonstrated that *Henna* has antibacterial and antifungal properties, and cures wound by hypoglycemic and anti-hyperglycemic effects [28,29].

The present study evaluated the effectiveness of compounds obtained from *A. capillus*, *C. molmol*, *Aloe vera*, and *Henna* when combined with petroleum jelly (Vaseline) for healing wounds in diabetic and non-diabetic rats. For this purpose, localization of TGFb1 and VEGF as important healing markers were evaluated by immunohistochemistry and the level of TGFβ1 and VEGF proteins were analyzed by Western blot analysis.

2. Materials and methods

2.1. Collection and preparation of herbal mixture

Fresh leaves of *Aloe Vera* (No.93) were collected from the botanic garden from Jondishapour Ahvaz University of Medical Sciences and were identified by department of horticulture in faculty of agriculture. Shoots of *A. capillus* were collected from Lorestan province in Iran (No. 1661). Fresh henna leaves were collected from Kerman city in Iran (KF 1408). The oleo gum resin of *C.molmol* has been obtained from Saudi Arabia. The origin of plant materials were systemically identified and approved in the herbarium of Shahid Chamran University of Ahvaz, Iran. After collection of plants, fresh leaves were washed twist and dried at 60 °C in oven. The dried leaves and resin of the *C. molmol* were then crumbled in a blender into a fine powder and then mixed in an equal part with Vaseline to a poultice.

2.2. Animals and grouping

This study was authorized by the Medical Ethics Committee of Ahvaz Jundishapur University of Medical Sciences with the number TRC-9204. Sixty adult male Wistar rats (200–220 g) have been used. The rats were housed separately and fed an unrestricted diet of food and water in a temperature-controlled (22–24 °C) room on a12 h light/dark cycle. The animals were divided into two non-diabetic and diabetic induced wound groups as described in Table 1.

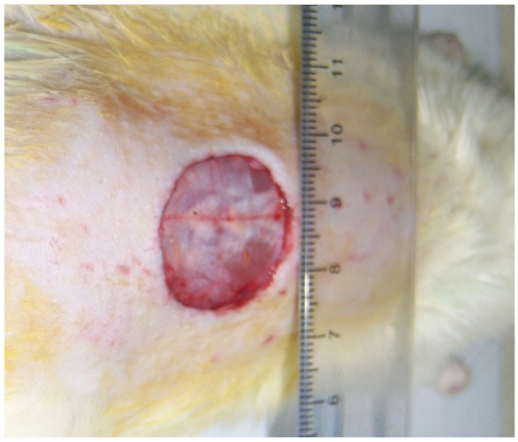

Fig. 1. Wound excised on the back of examined rat.

2.3. Induction of type 1 diabetes mellitus

After 12 h fasting, diabetes was induced in the test rats by intraperitoneal injection of 60 mg/kg of streptozotocin (Sigma-Aldrich; USA) in saline-sodium citrate buffer. The rats consumed 5% glucose solution all night to neutralize the hypoglycemia stimulated by the streptozotocin. Seven days after injection of streptozotocin, the blood glucose concentration of the rats was measured using a glucometer. One week post-injection, type 1 diabetes was identified in each rat by a blood glucose level that was consistently above 250 mg/100 ml. The diabetic rats exhibited typical symptoms of diabetes mellitus including polyuria, polyphagia, and weight loss. Throughout the study, blood glucose levels for each rat were tested every 3 days.

2.4. Wound model

The wound was created at beginning time on all testing rats. Each rat was anesthetized by intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). The hair on the back of rats was firstly shaved and the wound site was sterilized with 70% alcohol. The skin wound was established by excision of a circular region about 2 cm in diameter on the back of each rat (Fig. 1).

2.5. Tissue collection

At the day 7th, 14th, and 21th post-wounding, the rats were euthanized with chloroform. The wound and an edge of about

Table 1
Each group was classified into two subgroups including only Vaseline treatment and Vaseline plus herbal mixture. Each of subgroups contain five rats (n = 5). The gene expression was measured in periods of 7, 14, and 21 days after treatment with Vaseline or Vaseline and mixture.

Time (day)	Group I: non-diabetic		Group II: diabetic	
	Vaseline	Herbal mixture & Vaseline	Vaseline	Herbal mixture & Vaseline
7	5 rats	5 rats	5 rats	5 rats
14	5 rats	5 rats	5 rats	5 rats
21	5 rats	5 rats	5 rats	5 rats