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ORIGINAL ARTICLE

The Study of the Effect of Alcoholic Extract of *Triticum sativum*, on the Skin Wound Healing in Diabetic Male Wistar Rats

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KEYWORDS

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ABSTRACT: Diabetes mellitus is one of the most common diseases in the body's endocrine system. This disease is caused due to lack of cellular uptake of blood glucose due to a decrease of insulin secretion and insulin resistance in the body cells. Besides the development of diabetes, the more common underlying medical condition, such as foot deformities, foot ulcers, and infection, spreads. Wheat, with the scientific name "*Triticum sativum*", is a multiple therapeutic effect, including amplifiers, healer of wounds, stimulating and antiseptic, decreasing blood sugar and pressure, which can be beneficial in balancing sugar and insulin in the blood. Therefore, in this study, the effect of this plant was investigated on skin wound healing in diabetic rats. 48 male rats under the name of Wistar race weighing 200 to 220 grams were categorized into 4 groups and each group has 12 rats in the four categories: control group (without diabetes and treatment), sham (diabetic, treatment with Eucerin), experimental group 1 (diabetic without treatment), and experimental group 2 (diabetic and treatment with *Triticum sativum* ointment). Some scars were created with a length of 3 cm on the skin of the dorsal lumbar region in the rats of each group, and daily wound healing on these rats was observed. The results indicate that experimental group 2, compared to the rest of the other groups, has the best effect on wound healing. Based on the results of this study, probably, the extract of *Triticum sativum*, due to anti-steroid and anti-inflammatory attributes, can lead to improvement and decrease the length of the wound in the animal, after 21 days.

INTRODUCTION

Diabetes mellitus

More commonly referred to as "diabetes" -- a chronic disease associated with abnormally high levels of the sugar glucose in the blood. Diabetes is due to one of two mechanisms:

1. Inadequate production of insulin (which is made by the pancreas and lowers blood glucose), or
2. Inadequate sensitivity of cells to the action of insulin.

In diabetic patients due to diabetic complications (peripheral neuropathy, infection, peripheral vascular disease), foot injuries are common. Impaired wound healing in diabetic patients is a major clinical problem, which is associated with significant morbidity and mortality. Diabetic foot ulcers annually occur in 2 percent of people with diabetes. If in addition to diabetes, the central nervous system is also affected, leg and foot lesions, will increase up to 7 percent [3]. The high glucose environment also affects the interactions of growth factors such as insulin-like growth factors and vascular endothelial growth factors, thus resulting in poor re-epithelialization or angiogenesis [4]. Poor circulation,

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limited nutrients, and inflammation usually cause infections such as diabetic foot ulcers. Although antibiotics could be utilized to treat diabetic wounds, drug resistance is a common serious issue. Therefore, it is important to develop therapies from animal models to clinical trials to overcome those symptoms and shorten the diabetic wound healing process [5].

Wound healing is dynamic involving the communication of cells, growth factors, and cytokines. For instance, macrophages have various functions in the inflammation and proliferation phase, including the activation of inflammation, ECM synthesis, and supporting fibroblast proliferation [6, 7, 8, and 9]. Indeed, the cell-cell interaction is very complicated, and cell signaling requires ROS/RNS19. ROS/RNS not only inactivate bacteria but also react relatively quickly with proteins, cells, tissues, and even biological fluid. Hydrogen peroxide (H2O2) is a well-known ROS that participates in blood coagulation and wound contraction. It is also known for being the second messenger of tissue growth factor, platelet-derived growth factor, and vascular endothelial growth factor production. Owing to its high peroxidation activity, H2O2 is effective for bacteria inactivation. Furthermore, nitric oxide (NO) is another significant species in wound healing for angiogenesis, inflammation, and tissue remodeling. Further, it can stimulate endothelial cell proliferation and prevent cell apoptosis, thus promoting the formation of new blood vessels in angiogenesis [10].

Wheat, with the scientific name of *Triticum sativum*, belongs to the genus of *Triticum*, and the Poaceae family. Chemical changes occur in the grain during germination, causing an increase in its minerals and vitamins. The therapeutic effect of this plant is included amplifiers, a wound-healer, a stimulant, and antiseptic in blood sugar and pressure [11]. Extracts of *Triticum sativum*, like herbal medicine, are effective in the treatment of many diseases, especially in the treatment of metabolic diseases [12].

Therefore, the aim of the present study was the study of the effect of *Triticum sativum* on skin wound healing in diabetic rats.

MATERIALS AND METHODS

In this study, 48 adult male rats, with a weight range of 200-220 g were investigated from the Pasteur Institute, Karaj, and were kept in the animal house for 10 days to be adapted to the environmental conditions (temperature 22 ± 2°C and 12 hours of dark and 12 hours of the light cycle. The relative air humidity was between 40 to 60 percent, and they had free access to food and water). Procedures for keeping animals and their care were conducted in conformity with the Helsinki Declaration and guidelines for the care and use of laboratory animals. Committee of Animal Care and Use which is located in Damghan city at Islamic Azad University has approved this instruction.

The experimental method to create diabetes

After keeping the rats for 10 days using citrate buffer, streptozotocin was injected into 36 rats, with the dosage of 55 mg/kg, for each rat, 0.2 ccs, and after 72 hours, blood sugar levels in each mice was measured by glucometer. The blood sugar level in each of them was 296 to 579, which is indicative of diabetes.

Material injury and assessment of healing

The animals were anesthetized with the injection of appropriate doses of ketamine and xylazine, and afterward the back hairs of the animals were shaved so that, the skin was free of hair. Then, utilizing a surgical blade and considering all relevant principles, an incision was made with the length of 3 cm, and the depth of the epidermis, dermis, and hypodermis. The day of surgery was considered as zero-day and the rats were kept in a cage (for sanitation).

Preparation of ointment

For preparing the extract of *Triticum sativum*, 200 g of dried and ground plant was dissolved in 4x (1 liter) ethanol 70%. After 72 hours, the prepared extract was isolated by Rotary, and the extract was thick and then combined with three times volume of eucerin extract.

Treatment of rats

Wounds of the Control and the Experimental group 1 were not treated, but the Sham group, from the first day, on a daily basis, was treated with eucerin, and experimental group 2 was treated with the ointment of *Triticum sativum*.

DISCUSSION

For the treatment of diabetic ulcers, plants are a potential source of anti-diabetic drugs and healing [13]. Chemical compositions of plants, which reduce blood sugar, are glycoside, alkaloids, glycans, terpenes, mucilage, a polysaccharide, oils, vitamins, saponins, glycoproteins, peptides, the amino acids and proteins [14]. Since there is no effective drug for wound healing, investigation about herbs for this matter is essential [15, 16, 17].

In this research, the researcher used the extract of *Triticum sativum* for wound healing of diabetics. Nowadays many research studies have been done on the properties and effects of the plants in physiological and biochemical systems of the human body. *Triticum sativum* contains, on average, 7% fat, 60 percent carbohydrates, and about 13.5 percent nitrogen. It is fatty acids, including palmitic, oleic acid, and linoleic acid. Moreover, it also contains steroids, terpenes, vitamins A, B, C, D, E, and minerals such as selenium, potassium, fiber type Beta Glucotase [13]. The therapeutic effects of this plant are included amplifiers, a healer of wounds, stimulating and antiseptic, decreasing blood sugar and pressure which is helpful in balancing blood sugar and insulin levels [18]. Extracted *Triticum sativum*, is an effective herbal medicine in the treatment of many diseases, especially in the treatment of metabolic diseases [19]. Based on research results, the healing and the percentage of wound healing after 21 days are lower than the control group. Although with the time the wound has decreased, full recovery was not observed. On the other hand, in the group, that diabetic rats received the extract of *Triticum sativum*, the length of the wound fell to zero compared to Control and Sham groups after 21 days, and complete remission was observed in these rats. Great Solomon et al. have found that wound infections and chronic inflammation leads to a delay in wound healing [20]. However, anti-microbial and anti-inflammatory effects can be effective in accelerating healing. Sachin et al. have shown that alkaloids, due to their antioxidant activity, could have healing properties [21]. Moreover, Kumar et al. have stated that more flavonoids and terpenes increase wound contraction and epithelialization [22]. According to the results of this study, it is estimated that compounds of *Triticum sativum* cause decreasing lesion length in Experimental group 2 in comparison with Sham and Control group by passing time. Possibly by the development and proliferation of the epidermis layer of the skin, leads to faster wound healing in diabetics. As reducing angiogenesis and cell proliferation are the side effects of diabetes, the epithelialization rate mechanism can be considered as contributing factor for this matter [23]. Plants with anti-inflammatory properties have a high level of flavonoids [24]. It can be concluded that, *Triticum sativum*, due to its anti-inflammatory effect, is flavonoids. Its compounds have antioxidant activity and neutralize free radicals, which improve diabetes, and as a result, will be diabetic wound healing.

According to the abovementioned explanations, because of an anti-steroid attribute of wheat germ, the process of recovery will accelerate, and the length of the animal's wound will reduce, after 21 days. The general conclusion can be stated that *Triticum sativum*, makes wound healing by increasing the percentage of wound healing and reducing lesion length. Since the prevalence of diabetes in our communities is increasing, identifying effective methods for wound healing and reduction in amputation can improve the quality of life and reduces health care costs.

RESULTS

To evaluate wound healing in Control, Sham, Experimental 1 and Experimental 2 groups, in 3.5, 7, 14, and 21 days, lesion length was measured by caliper and the results were analyzed using SPSS and Duncan test and ANOVA, in which, the results can be seen in below.

The Mean±SD percentages of wound healing in 3.5, 7, 14, and 21 days are depicted in Table1. Results show that the percentage of wound healing at day 3.5 does not have a significant difference between Control, Experimental 1 and Experimental 2 groups (Figure 1). According to Figure 2, on day 7, the percentage of wound healing in the experimental 1 group was significantly decreased in comparison with Control and Experimental 2 groups ($P \leq 0.05$), and the Experimental 2 group has a bigger percent in comparison with the Control group ($P \leq 0.05$). At the day 14, the percentage of wound healing in Experimental 1 group, compared to the Control group, shows a significant decrease ($P \leq 0.05$), and in the Experimental 2 group shows a significant increase in comparison with the Control group ($P \leq 0.05$) (Figure 3). As it is shown in Figure 4, the percentage of wound healing in experimental group 1 shows a significant reduction on day 21 ($P \leq 0.05$) in comparison with the control group and experimental group 2.

Microscopic studies showed that during 21 days, the process of wound healing in the Experimental 2 group, which was treated with *Triticum sativum*, has faster than Control and Experimental 1 groups (Figures 5 to 7).

Table 1. Mean±SD of wound healing for different groups

Day **Group**	**3.5**	**7**	**14**	**21**
Control	19.57±4.07	62.71±1.82	95.12±1.35	99.94±0.03
Sham	17.01±3.65	53.66±2.24	85.41±1.56	98.09±0.19
Exp1	17.04±3.52	54.06±2.49	85.42±1.56	98.09±0.19
Exp2	19.95±1.37	69.57±0.42	96.82±0.55	100.00

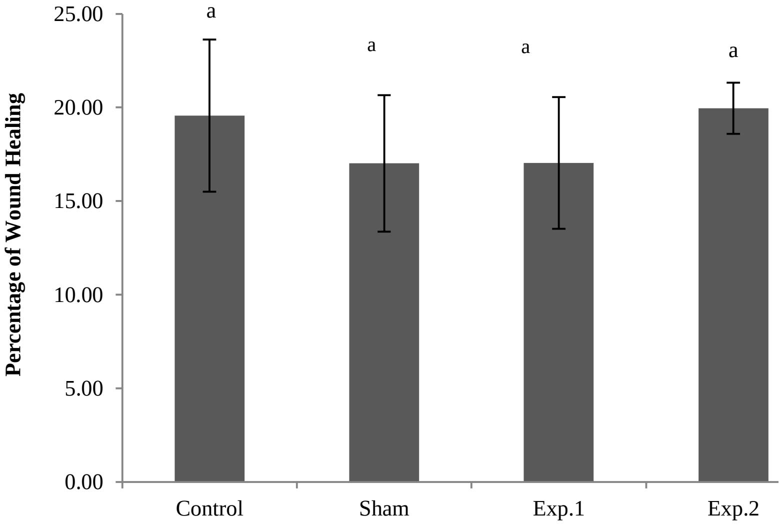

Figure 1. Mean ± standard deviations, percentage of wound healing in experimental groups, at the day 3.5

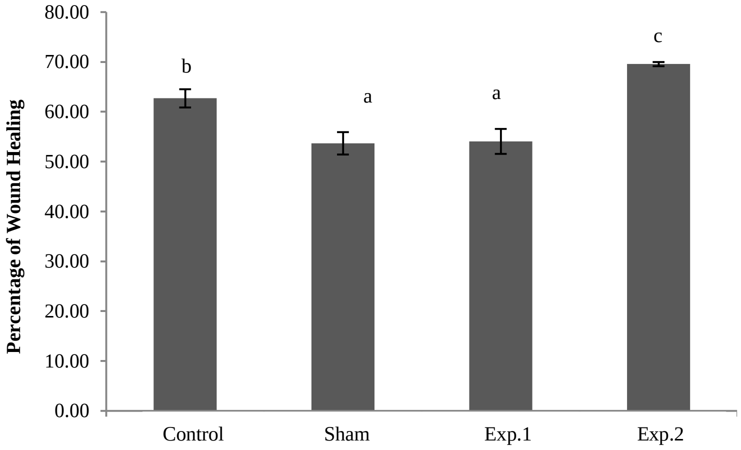

Figure 2. Mean ± standard deviations, percentage of wound healing in experimental groups, at the day 7.

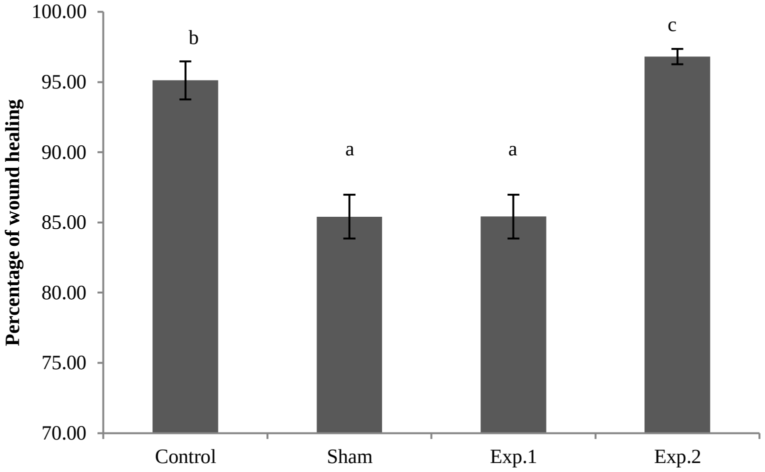

Figure 3. Mean ± standard deviations, percentage of wound healing in experimental groups, at the day 14.

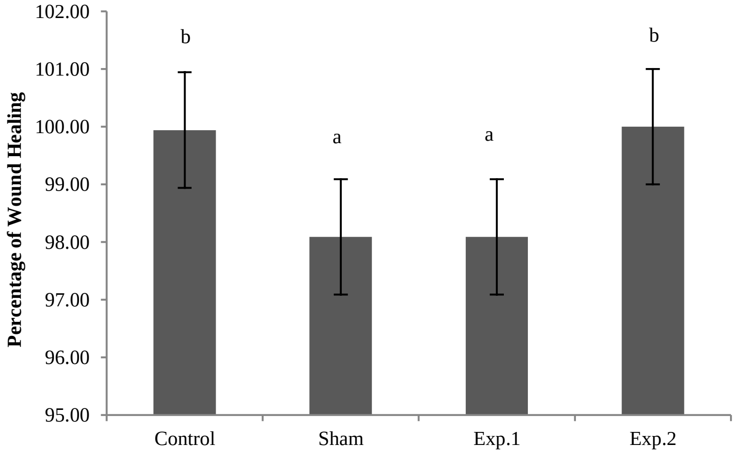

Figure 4. Mean ±, standard deviations, percentage of wound healing in experimental groups, at the day 21.

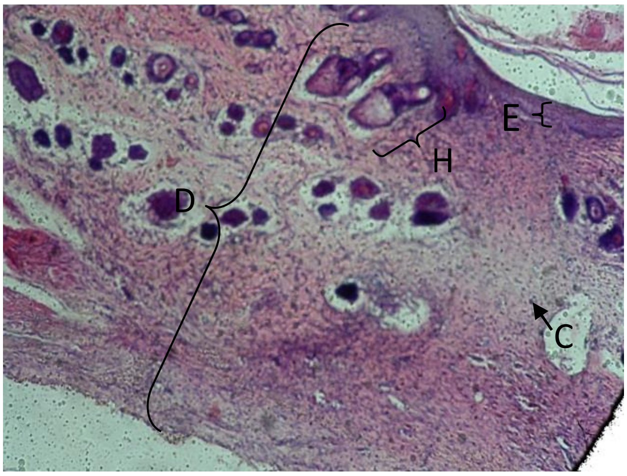

Figure 5. Microscopic study of wound healing, *10 magnification in experimental group 2, on day 14.
E: epidermis, C: collagen, D: dermis, H: hair shaft

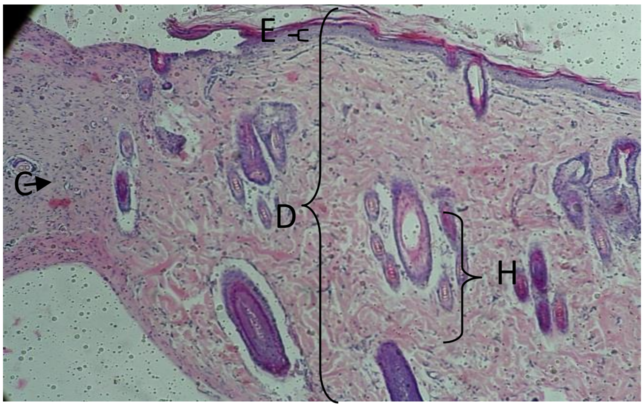

Figure 6. Microscopic study of wound healing, *10 magnification in experimental group 1, on day 21.
E: epidermis, C: collagen, D: dermis, H: hair shaft

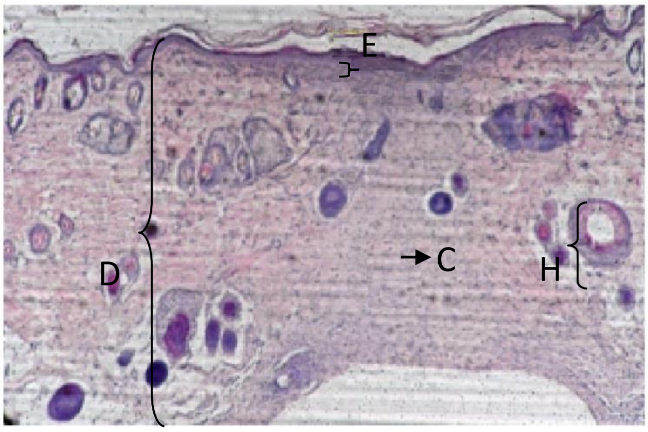

Figure 7. Microscopic study of wound healing, *10 magnification in control group, on day 21.
E: epidermis, C: collagen, D: dermis, H: hair shaft

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Conflict of interests

The authors declare no conflict of interest.

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