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**Original Article**

# Effectiveness of oral administration of Fenugreek seed aqueous extract on excision wound healing in diabetic rats

**Seyyed Rezgar Hoseini [1], Mohammad Reza Farahpour [2*], Sakineh Nouri Saeidlou [3]**

*[1] Department of Pathobiology, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran*
*[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran*
*[3] Food and Beverage Safety Research Center, Urmia University of Medical Science, Urmia, Iran*

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ABSTRACT

Diabetes is one of the most common glandular diseases. Delay in wound healing process is known as a major challenge in these patients. Fenugreek is known to have therapeutic effects. Thus, the present study was conducted to investigate the oral administration of fenugreek seed aqueous extract (FSE) on wound healing in the experimental animal model. An excisional model was created on 24 rats. Streptozotocin was administrated in order to induce the diabetes. A surgery was performed and a circular wound with a diameter of 6 mm was created on the dorsal surface of each rat. Animals were divided into 4 groups including non-diabetic, diabetic control and diabetic treated rats, with oral administration of different doses of FSE (200 and 400 mg/kg). The wound area, histological parameters, blood glucose and insulin levels were measured on days 3, 7 and 16. The results showed that the wound area and blood glucose were significantly decreased in FSE-treated animals in comparison with diabetic control from day 7 ($P<0.05$). It can be suggested to use oral fenugreek seed aqueous extract on improved the blood sugar and wound healing in diabetic rats.

## اثربخشی تجویز خوراکی عصاره آبی بذر شنبلیله بر التیام زخم برشی در موش های صحرایی دیابتی

**سید رزگار حسینی [۱]، محمدرضا فرهپور [۲*]، ، سکینه نوری سعیدلو [۳]**

*[۱] گروه پاتوبیولوژی، دانشکده دامپزشکی، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه، ایران*
*[۲] گروه علوم درمانگاهی، دانشکده دامپزشکی، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه، ایران*
*[۳] مرکز تحقیقات ایمنی نوشیدنی و غذا، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران.*

**چکیده**

دیابت، یکی از بیماری های غده ای در جهان است و التیام زخم را به تأخیر می اندازد. شنبلیله، دارای اثرات دارویی است. بنابراین این مطالعه با هدف بررسی کارایی تجویز خوراکی عصاره آبی بذر شنبلیله بر التیام زخم باز در مدل حیوانی آزمایشی دیابت انجام شد. استرپتوزوتوسین برای القای دیابت تجویز شد. یک زخم برشی با اندازه ۶ میلیمتر روی ناحیه پشتی ۲۴ موش صحرایی ایجاد شد. حیوانات به ۴ گروه غیردیابتی، کنترل دیابتی و دیابتی تجویز خوراکی شده با ۲۰۰ و ۴۰۰ میلی گرم/کیلوگرم از عصاره شنبلیله تقسیم شدند. اندازه ناحیه زخم، پارامترهای بافت شناسی و غلظت سرمی انسولین و گلوکز خون در روزهای ۳، ۷ و ۱۶ بررسی شدند. نتایج این مطالعه نشان داد که تجویز عصاره آبی بذر شنبلیله بطور معنی داری ناحیه زخم و غلظت گلوکز را در مقایسه با گروه کنترل از روز ۷ کاهش داد ($P<۰/۰۵$). در مجموع، تجویز خوراکی عصاره آبی بذر شنبلیله قند خون را کاهش داده و باعث بهبود التیام زخم در موش های صحرایی دیابتی شد.

**واژه های کلیدی:** زخم برشی، التیام، بذر شنبلیله، موش صحرایی دیابتی

* Corresponding author: mrf78s@gmail.com

## INTRODUCTION

Diabetes mellitus is one of the most important diseases in the developed countries which has several social and economic consequences for patients. Under normal circumstances, the wound healing includes stages of rapid hemostasis, inflammatory phase, proliferation and migration to the wound site, appropriate angiogenesis, re-epithelialization and proper biosynthesis, cross-linking, and maturation of collagen (maturation phases) [1-3]. Diabetes mellitus delays wound healing due to hyperglycemia, oxidative stress vascular insufficiency, and microbial infections [4]. Diabetes increases the duration of the inflammatory phase and delays the proliferative phase including cellular infiltration, angiogenesis and attenuation of wound contraction and closure [5]. Hyperglycemia delays the wound healing because of increased oxidative stress and intensive inflammation [6-8]. Synthetic hypoglycemic agents have been faced with limitations because of serious side effects. For long years, the diabetics have been treated with several medicinal plants or their extracts [7]. Fenugreek (*Trigonella foenum-graecum*) is broadly applied in order to prepare the Ayurveda and also known to have antiulcer, hypocholesterolemic [9, 10] and hypoglycemic effects [11]. Fenugreek seeds have some polysaccharides such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens. Saponins can show steroidal effects which can decrease inflammation in the body [12]. Other bioactive constituents of fenugreek are including mucilage, volatile oils, flavonoids, amino acid and alkaloids. The other active ingredients found in fenugreek is 4-hydroxyisoleucine [13]. It was hypothesized that fenugreek can improve blood sugar and help to accelerate the wound healing. Thus, this study was conducted to evaluate the effects of oral administration of fenugreek seed aqueous extract (FSE) on blood sugar and wound healing in excisional wound in diabetic rats.

## MATERIALS AND METHODS

### *Preparation of aqueous extracts of fenugreek*

Fenugreek seeds were purchased from medicinal plants market in Mahabad city. The seeds washed and dried and the obtained powder was extracted. The achieved extract was concentrated by distillation apparatus. The obtained solution was filtered and final solution was dried in 25∘C and kept in -4∘C until the ointment was prepared.

### *Experimental animals*

Twenty-four Wistar male rats, 200±10 g, were prepared and all the used procedures were approved by the Ethical Standard Committee, Islamic Azad University, Urmia Branch (No. 1209). Animals were kept in individual cages in order to adapt to a new environment. Animals were kept in the optimum temperature (22±3∘C), humidity (60±5%) and lighting cycle (12h light: 12h darkness). Animals had ad libitum access to water and feed.

### *Induction of diabetes*

In order to induce the diabetes, streptozotocin (STZ) dissolved in citrate buffer (pH=4.5) was intraperitoneal administrated in each rat (40 mg/kg). Blood sugar was measured by glucometer (Norditalia, Italy) by tail vein. Animals with glucose >300 mg equivalent was considered as diabetic ones [1, 2].

*Induction of wound*

General anesthesia was induced by intraperitoneal administration of xylazine hydrochloride 2% (5 mg/kg) and ketamine hydrochloride 5% (50 mg/kg) [1, 2]. After induction of anesthesia, rats were positioned on surgical table and a circular wound (6 mm diameter) was created in the space between the two shoulders. After induction of wound, all the rats were divided into 4 groups (n=6) including two non-diabetic and diabetic control groups that administrated 300 mg/kg oral normal saline, and two treated groups which diabetic rats were administrated 200 and 400 mg/kg oral fenugreek seed aqueous extract (FSE). The extract was the same in both doses but different concentrations were used.

*Determination of blood glucose and insulin*

The blood samples were collected from the tail vein on days 3, 7 and 16 after the STZ administration and the fasting blood glucose levels were measured by a glucometer (Accu check, activate Blood Glucose Meter System, Int. Medical Equipment Diabetes Care, Berlin, Germany). The level of insulin was measured by Pars Azmoon commercial kits (Tehran-Iran).

*Wound closure*

A transparent paper was used to evaluate the surface areas of wounds. This site was calculated by the impression of a graph sheet [10, 11]. The percentage of wound closure was calculated by the initial and final sites drawn on glass slides during the experiments, as follows;

Wound closure (%) = Wound area on day zero -Wound area on day X/wound area on day zero × 100. In this formula X = day post-injury (3rd, 7th and 16th days).

*Histology*

In order to the microscopic evaluation, two rats were randomly euthanized, and a sample of tissues was removed, on day 16. A part of the wound was separated and tissue samples were fixed in 10% of the neutral buffered formalin solution. In order to evaluate the histological spots, samples were processed into 5-μm-thick paraffin sections [1, 2]. The formation of granulation tissue, new vessels formation and re-epithelialization were examined by the usual method for trichrome staining.

*Statistical analysis*

Our data were analyzed by SPSS18 software (SPSS Inc., Chicago, IL, USA) and by the ANOVA and Tukey post hoc tests. We used ANOVA test in order to analyze the differences among the three groups based on quantitative variables such histological parameters. The Tukey test was used in order to compare among a set of means. All tests of statistical significance were two-tailed, with the degree of significance at $P<0.05$.

## RESULTS

*Blood glucose and insulin*

As results indicate, oral administration of FSE, especially higher doses, significantly decreased blood glucose (Fig. 1-A) and increased insulin levels (Fig. 1-B) from 7th to 16th days ($P<0.05$). The results showed that glucose concentration was significantly lower in the rats treated with 400 mg/kg of FSE compared with the ones treated with 200 mg/kg of FSE in all days. In addition, insulin concentration was significantly higher in the rats treated with 400 mg/kg of FSE compared with those treated with 200 mg/kg of FSE in all days.

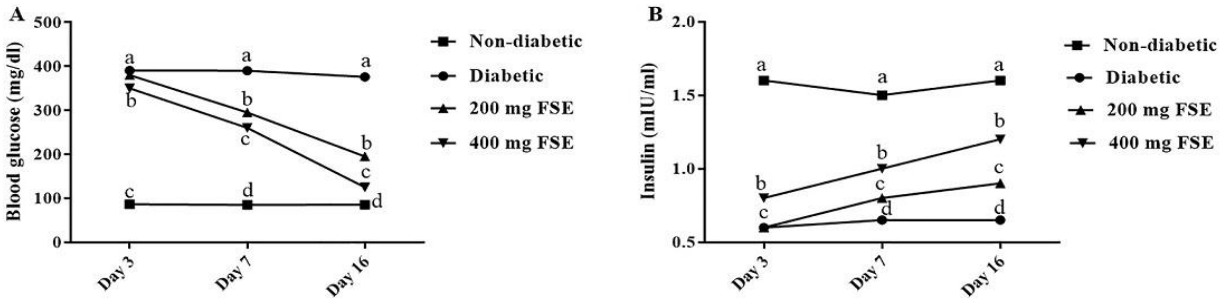


**Figure 1:** Effects of treatments on blood glucose (mg/dl) and insulin (mIU/ml) levels in the different days. Superscripts (a-d) show significant differences in same day among groups.

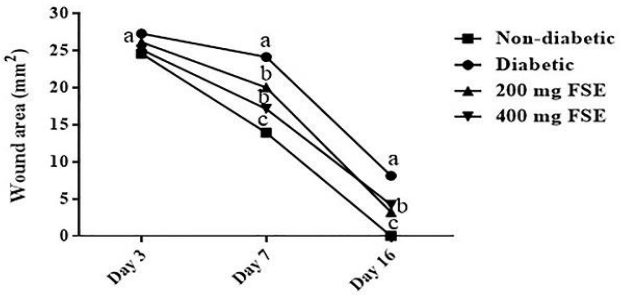


**Figure 2:** Effects of treatments on wound area ($mm^2$) in the different days. Superscripts (a-c) show significant differences in same day among groups.

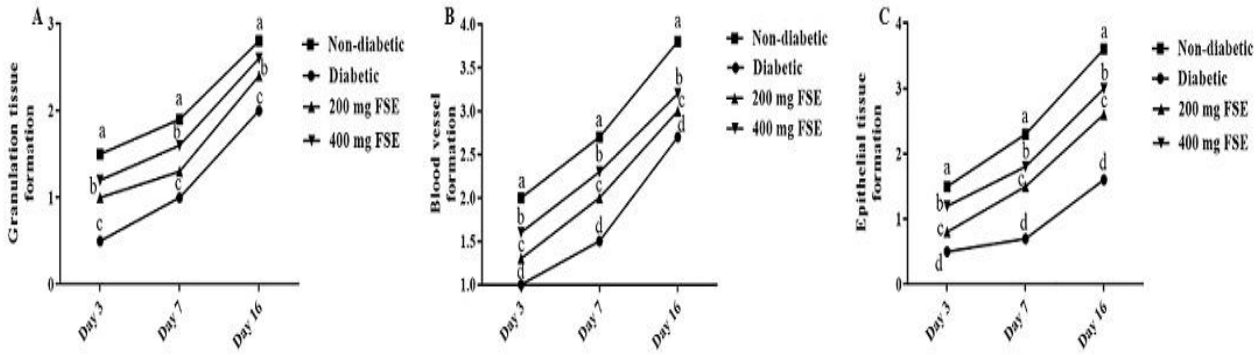


**Figure 3:** Effects of treatments on granulation tissue formation (A), blood vessel formation (B) and epithelial tissue formation (C) in the different days. Superscripts (a-c) show significant differences in same day among groups.

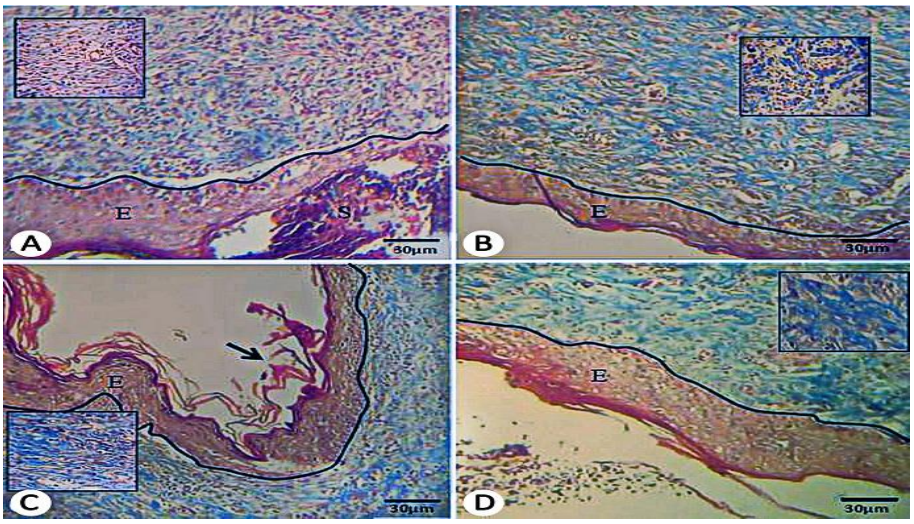


**Figure 4:** Histopathological section from wound area on day 16; (A) Diabetic control group: incomplete epidermis (E) under remained scab (S) accomplished with faint collagen condensation. (B) 200 mg FSE-treated group: complete migration of the epidermal cells (E) with collagen generation (see inset). (C) 400 mg FSE-treated group: the epidermis (E) is presented with complete layers (arrow) and condensed collagen. (D) Non diabetic control-treated group: complete epidermis (E) with well-formed collagen in dermis (see inset). Masson's trichrome.

*Wound area*

Our findings for the wound area are shown in Figure 2. The wound area was significantly reduced in FSE-treated animals, especially 400 mg/kg of FSE, in comparison to diabetic control animals ($P<0.05$). The results showed that the wound area was significantly lower in the rats treated with 400 mg/kg of FSE compared with those treated with 200 mg/kg of FSE in all the days.

*Histological analysis*

Our data (Fig. 3A-C) showed that oral administration of FSE, especially higher levels, significantly improved granulation tissue formation, blood vessel formation and epithelial tissue formation on days 3, 7 and 16 after induction of wound in comparison to diabetic control ones (Fig. 4) ($P<0.05$). The results showed that the best result for wound healing was seen in the rats treated with 400 mg/kg of FSE compared with ones treated with 200 mg/kg of FSE in all days.

## DISCUSSION

Results showed that oral administration of fenugreek aqueous extract significantly accelerated wound healing process. Fenugreek extract is known to have some compounds such as saponins. Saponins can produce steroidal effects that could reduce inflammation in the body [14]. It was reported that fenugreek releases anti-inflammatory substance into wound region and decreases inflammation [14]. In addition, antimicrobial properties of fenugreek may increase its anti-inflammatory responses. A study has shown that flavonoids and triterpenoids may promote the wound-healing process because of its antimicrobial properties [15]. Thus, it shortens inflammatory phase and promotes proliferative phase that is a proof for accelerating the wound healing process. Results indicated that FSE improved granulation tissue formation and epithelial tissue formation. Angiogenesis supplies the essential needed nutrients and oxygen during wound healing process and also accelerates granulation tissue formation [16]. It can be attributed to some of chemical compounds present in the extract that can have angiogenic and mitogenic potentials [13]. In addition, fenugreek is known to have

antioxidant properties which can accelerate wound healing [17]. Thus, oral administration of FSE accelerates wound healing by promoting the proliferative phase. Oral supplementation of FSE decreased the levels of glucose and insulin. Hasan and Rahman (2016) have reported that fenugreek extract decreases glucose metabolism by increasing adipocyte differentiation and preventing inflammation in adipose tissue [11]. It is accepted that Streptozotocin enhances the levels of glucose and excretes it [11]. Gu et al. (1997) have reported that Streptozotocin destroys pancreatic β-cells and decreases capability of insulin secretion [18]. Some studies have shown that administration of Streptozotocin enhanced glucose concentration and decreased insulin concentration in rats [19, 20]. Montilla et al. (2005) have reported that diabetes caused to induce the injuries in tissues and brain and also hyperglycemia [21]. Insulin not only regulates glucose intake by glucose transporters-1 (GLUT-1) but it also enhances the total amounts of GLUT1 and its expression in the plasma membrane of the basal cells in skin [22]. Topical administration of insulin up-regulated GLUT-1 by improving wound healing in diabetic rats [23]. Our findings showed that as levels of insulin increases, glucose levels decrease in FSE groups. It seems that FSE decreases glucose levels by increasing levels of insulin which helps to transport glucose into cell. On the other hand, Velander et al. (2008) have reported that the delayed wound healing in the experimentally-induced diabetes is not induced by just local high-glucose concentration. It can be stated that FSE helps to transport glucose into cell and to improve wound healing [24].

## CONCLUSION

Due to its reasonable price, convenient use, low side effects, and according to the results of this study and other studies, Levamisole hydrochloride drug can be a suitable option to strengthen the immune system in birds.

## ACKNOWLEDGMENTS

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## ETHICS

Approved.

## CONFLICT OF INTEREST

None declared.

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