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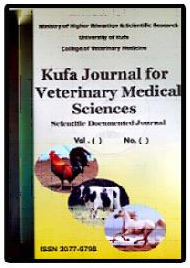

Effects of Fenugreek (Trigonella foenumgraecum) Oil On Matrix Metalloprotease type 2 (MMP2) and Fibroblast growth factor type 2 (FGF2) expression during Wounds Healing Process in Diabetic Male Wister Rat

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Abstract

Background: Wound healing is a complicated process occurred to reform the integrity and homeostasis of wounded tissue, there is evidence that fenugreek induce and/or enhance the healing process in diabetic in male Wister rats.

Aim: To investigate the effects of Fenugreek on levels of matrix metalloprotease type 2 (MMP2) and (FGF2) levels during wound healing process of diabetic male Wister rats via immunohistochemistry techniques.

Methods: Thirty diabetic male Wister rats were divided into two main groups each group 15 rats, namely group D (diabetic rats without treatment) and group FD (diabetic rats treated with 10% fenugreek oil applied locally on wound area). Five rats were euthanized from each group at week two and week three of experimental period. MMP2 and FGF2 expression levels were measured using immunohistochemistry technique.

Results: Fenugreek oil showed the ability to decrease the expression of MMP2 in 10% fenugreek oil treated diabetic rats during week two and week three of wound healing process compared with diabetic rats without treatment. However, Fenugreek oil stimulated high expression of FGF2 in 10% fenugreek oil treated diabetic rats during experimental period compared with diabetic rats without treatment.

Conclusion: The results of the present study revealed that fenugreek oil treatment has the ability to reduce the expression of MMP2 and elevate FGF2 expression in 10% fenugreek oil treated diabetic rats compared with non-treated diabetic rats.

Key words: ***diabetes mellitus, MMP2, FGF2, fenugreek oil, Wister rats***.

تأثيرات زيت الحلبة على تعبير Matrix Metalloprotease 2 (MMP2) و Fibroblast (FGF2) Growth Factor 2 اثناء عملية التأم الجروح في ذكور جرذان الوستير المصابة بداء السكري

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الملخص:
المقدمة: التئام الجروح هو عملية معقدة حدثت لإصلاح سلامة واستتباب الأنسجة المصابة ، وهناك دليل على أن الحلبة تحفز و / أو تعزز عملية الشفاء في مرضى السكري في ذكور الجرذان الوستر.
الهدف: دراسة تأثير الحلبة على مستويات ومستويات Matrix Metalloprotease 2 (MMP2) و Fibroblast Growth Factor 2 (FGF2) أثناء عملية التئام الجروح لدى ذكور جرذان الوستر المصابة بداء السكري من خلال تقنيات IHC.
طرق البحث: تم تقسيم ثلاثين من ذكور جرذان الوستر المصابة بداء السكري إلى مجموعتين رئيسيتين كل مجموعة 15 جرذًا ، وهي المجموعة D (الفئران المصابة بداء السكري دون علاج) والمجموعة FD (الفئران المصابة بداء السكري التي عولجت بزيت الحلبة بنسبة 10٪ الموضع على منطقة الجرح). تم قتل خمسة فئران من كل مجموعة عند الاسبوع الثاني و الاسبوع الثالث الفترة التجريبية. تم قياس مستويات التعبير MMP2 و FGF2 باستخدام تقنية IHC .
النتائج: أظهر زيت الحلبة قدرته على تقليل تعبير MMP2 في الفئران المصابة بداء السكري المعالجة بزيت الحلبة بنسبة 10٪ خلال الأسبوع الثاني والأسبوع الثالث من عملية التئام الجروح مقارنة بالفئران المصابة بداء السكري بدون علاج. ومع ذلك ، فقد حفز زيت الحلبة التعبير العالي لـ FGF2 في من الجرذان المعالجة بزيت الحلبة بنسبة 10٪ و المصابة بداء السكري خلال فترة التجربة مقارنة بالفئران المصابة بداء السكري دون علاج.
الخلاصة: أظهرت نتائج الدراسة الحالية أن العلاج بزيت الحلبة له القدرة على تقليل تعبير MMP2 ورفع تعبير FGF2 في الجرذان المصابة بداء السكري و المعالجة بزيت الحلبة بنسبة 10٪ مقارنة بالفئران المصابة بداء السكري.
الكلمات الأساسية: *داء السكري ، MMP2، FGF2 ، زيت الحلبة ، الفئران الوستر*

Introduction

A wound is defined as a disruption in the continuity of the epithelial lining of the skin or mucosa resulting from physical or thermal damage. According to the duration and nature of healing process, the wound is categorized as acute and chronic [1]. Wound healing is a dynamic, complex process that leads to the re-establishment of tissue integrity and homeostasis [2].

This process is coordinated by a complicated signaling mechanism that involves various enzymes, growth factors, cytokines, and chemokines. The process of wound healing occurs in three phases such as inflammatory phase, proliferative phase and remodeling phase [3].

The diabetes mellitus is very serious chronic disease in public health, estimated 476.0 million cases of diabetes worldwide in 2017 [4]. Diabetes mellitus, has two principal clinical forms identified as types 1 and 2. The former is a condition in which by autoimmune mechanisms pancreatic beta cells are eventually destroyed with an absolute insulin deficiency [5]. Diabetes mellitus characterized by chronic hyperglycemia with alteration in cellular homeostasis, leading to massive vascular damage and multi-organ dysfunction. Diabetic patients' risk both microvascular and macrovascular complications: the former result from damage to retinal, renal, and neural tissues [6]. Hyperglycemia in diabetes leads to the formation of heterogeneous moieties called advanced glycation end products (AGEs), it is one of the mechanisms that cause impaired wound healing process in diabetic patients [7]. Glycation of proteins such as enzymes, growth factors and other vital proteins leading to impairment of these proteins function, causing several organ dysfunctions in biological processes and organs. The impaired wound healing process one of these process that affected in protein glycation: including several important growth factors such as FGF2, EGF and VEGF [8].Impaired wound healing process associated with diabetes disease and considered one of the most dangerous consequences that threaten the life of diabetic patient, where the impairment of wound healing process led to death of body tissue causing a wet gangrene condition, especially in lower and upper extremities of diabetic patients [9].The MMP2 is a collagenase enzyme able to degradation of collagen fiber [10]. In non-diabetes cases, the MMP2 enzyme levels increased and continued during first week of wound healing process and will be minimized to absent after one week. The role of MMP2 enzyme to remodeling the wound tissue, preparing it for further healing process [11]. The MMP2 paly a main role in impaired wound healing in diabetic patients. In diabetes, the levels of MMP2 continuous increased during all wound healing process, causing degradation of any collagen fiber deposition delaying the wound healing process [12].

Fibroblast growth factor [FGF] 2, also called basic FGF, is a member of a large FGF family of structurally related proteins that bind heparin sulfate and modulate the growth, differentiation, migration and survival of a wide variety of cell types [13]. FGF2 have the ability to activate fibroblasts and other mesoderm-derived cells, including vascular endothelial and smooth muscle cells, osteoblasts and chondrocytes [14]. Administration of recombinant FGF2 to skin wounds enhance and accelerate acute and chronic wound healing [15]. The previous researches documented that FGF2 expression decreased wound healing of diabetic patients [16]. Glycation of FGF2 due to hyperglycemia is documented in diabetes, where glycated FGF2 led to impairment in FGF2 function and reduction its expression, effecting on collagen fibre production and deposition [17].

Materials and Methods

Thirty diabetic male Wister rats were divided into two main groups each group 10 rats, namely group D (diabetic rats without treatment) and group FD (diabetic rats treated with 10% fenugreek oil applied locally on wound area). One-week post-injection of Alloxan the rats were fasted for one day then the peripheral blood was withdrawn from rats to test the glucose levels to confirm the diabetes mellitus induction. The fasting blood glucose test results showed the blood glucose of all tested rats was at mean of 238.6 mg/dL, indicating the hyperglycemia in rats of the current study [18].

At day one of the experimental period, the dorsal inter-scapular region was shaved and the surgical wound was produced in this region for all rats by removing the whole layer of skin at circle shape (2cm in diameter) under general Anesthesia. Rats of both groups (five rats each) were euthanized at week two and week three of experimental period.

Immunohistochemistry

Skin samples were fixed in 10% formalin for 48 hour, processed, embedded in paraffin and section at 4 µm thickness. The immunohistochemistry was performed using Dako EnVision detection immunohistochemistry kit (Envision FLEX, Dako, K8000, Denmark) and as per manufacturer's instruction. Anti-Matrix Metalloprotease type 2 (MMP2) primary antibody (monoclonal Mouse Anti- MMP2 Antibody: sc-13595, SANTA CRUZ BIOTECHNOLOGY, USA) and Anti Fibroblast growth factor type 2 (FGF2) primary antibody (monoclonal Mouse Anti-FGF2 Antibody: sc-74412, SANTA CRUZ BIOTECHNOLOGY, USA) were used for detection the expression of MMP2 and FGF2. The immunohistochemistry score results were analyzed suing T test and Mann Whitney non-parametric-test methods to compare between D and FD groups.

Results and Discussion

The MMP2 is a collagenase enzyme able to degradation of collagen fiber [11, 12]. In non-diabetes cases, the MMP2 enzyme levels increased and continued during first week of wound healing process and will be minimized to absent after one week. The role of MMP2 enzyme to remodeling the wound tissue, preparing it for further healing process [11, 12]. However, in diabetes cases, the levels of MMP2 continuous increased during all wound healing process, causing degradation of any collagen fiber deposition delaying the wound healing process. [13, 19]

Table 1: Means of FGF2 and MMP2 expression of D and FD groups

Groups	FGF2				MMP2			
	Two weeks		Three weeks		Two weeks		Three weeks	
	Mean	± S.D	Mean	±S.D	Mean	±S.D	Mean	±S.D
FD	2.6 [a]	±0.55	3.2 [a]	±0.45	1.4	±0.55	0.6	±0.55
D	1.2	±0.45	1.4	±0.55	3 [a]	±0.00	2.6 [a]	±0.55

[a] Significant overexpression of FGF2 between FD and D. [a] significant overexpression of MMP-2 between D and FD.

The fenugreek showed an anti-tumor activity when examined in cancer researches, where the fenugreek decreased the expression of MMP2 in Glioblastoma Cell. [20] Also, the fenugreek showed the ability to decrease the MMP2 levels in renal diabetic rats. [21]

According to MMP2 results score of the present study, the fenugreek oil showed the ability to decrease the expression of MMP2 in diabetic rats (Table 1) at week two wound (Fig. 1) and week three (Fig. 1) wound samples compared with diabetic rats without treatment, where MMP2 enzyme levels continuous increased (Fig. 1).

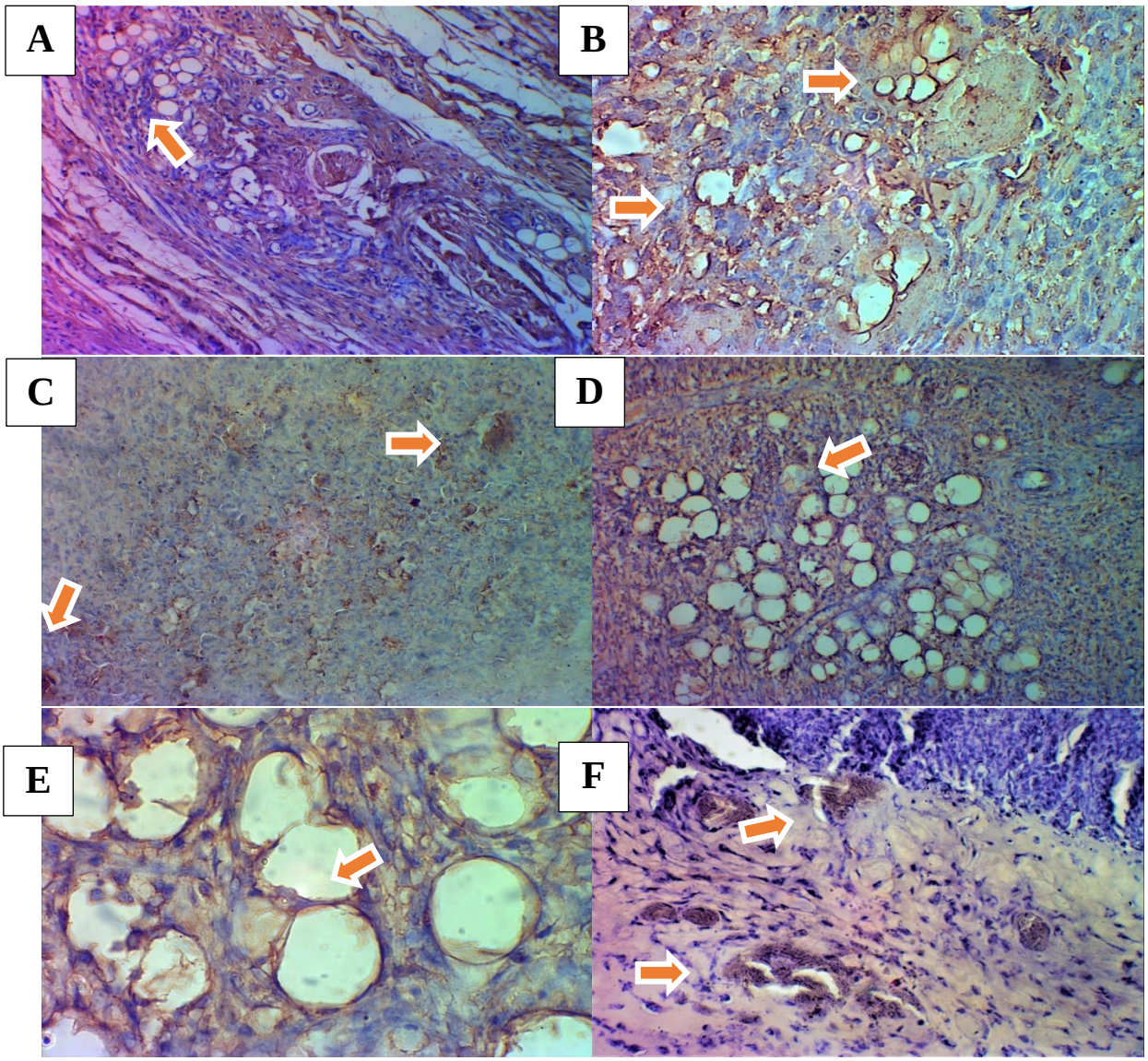

Figure 1: Photomicrograph of MMP2 expression of FD and D groups.
A&B/ The overexpression of MMP2 enzyme in group D rat (arrows) was observed in dermis layer of wound area with presence of spaces in affected area due to the activity. **C/** The expression of MMP2 enzyme (arrows) in group FD rat was low compared with the MMP2 expression in group D rat. **D&E/** The overexpression of MMP2 enzyme in group D rat (arrows) was observed in dermis layer of wound area with presence of spaces in affected area due to the activity. **F/** The expression of MMP2 enzyme (arrows) in group FD rat was low compared with the MMP2 expression in group D rat. **IHC: DAB and Mayer's hematoxylin. A, C, D and F: x100 and B&E: x400.**

FGF-2 is a potent molecule stimulates smooth muscle cell growth, wound healing, and tissue repair. [14, 22, 23] Administration of recombinant FGF2 to skin wounds enhance and accelerate acute and chronic wound healing. [14, 15, 24, 25] The previous researches documented that FGF2 expression decreased wound healing of diabetic patients. [17, 26]

Hyperglycemia in diabetes leads to the formation of heterogeneous moieties called advanced glycation end products [AGEs], it is one of the mechanisms that cause impaired wound healing process in diabetic patients. [7] Glycation of FGF2 is documented in diabetes, where glycated FGF2 led to impairment in FGF2 function and reduction its expression. [8, 27] Fenugreek seed extract showed an anti-glycation effect in previous studies. [28] The FGF2 expression score results of the present study showed a significant overexpression in diabetic rats treated with 10% fenugreek oil at week two (Fig. 3) and week three (Fig. 4) samples compared with diabetic non-treated diabetic rats (Fig. 3, 4) at same period of sample collection, due to anti-glycation effects led to prevent the glycation effects of hyperglycemia on FGF2.The FGF2 expression score results of the present study showed a significant overexpression in diabetic rats treated with 10% fenugreek oil compared with diabetic non-treated diabetic rats, due to anti-glycation effects led to prevent the glycation effects of hyperglycemia on FGF2 (Table 1)

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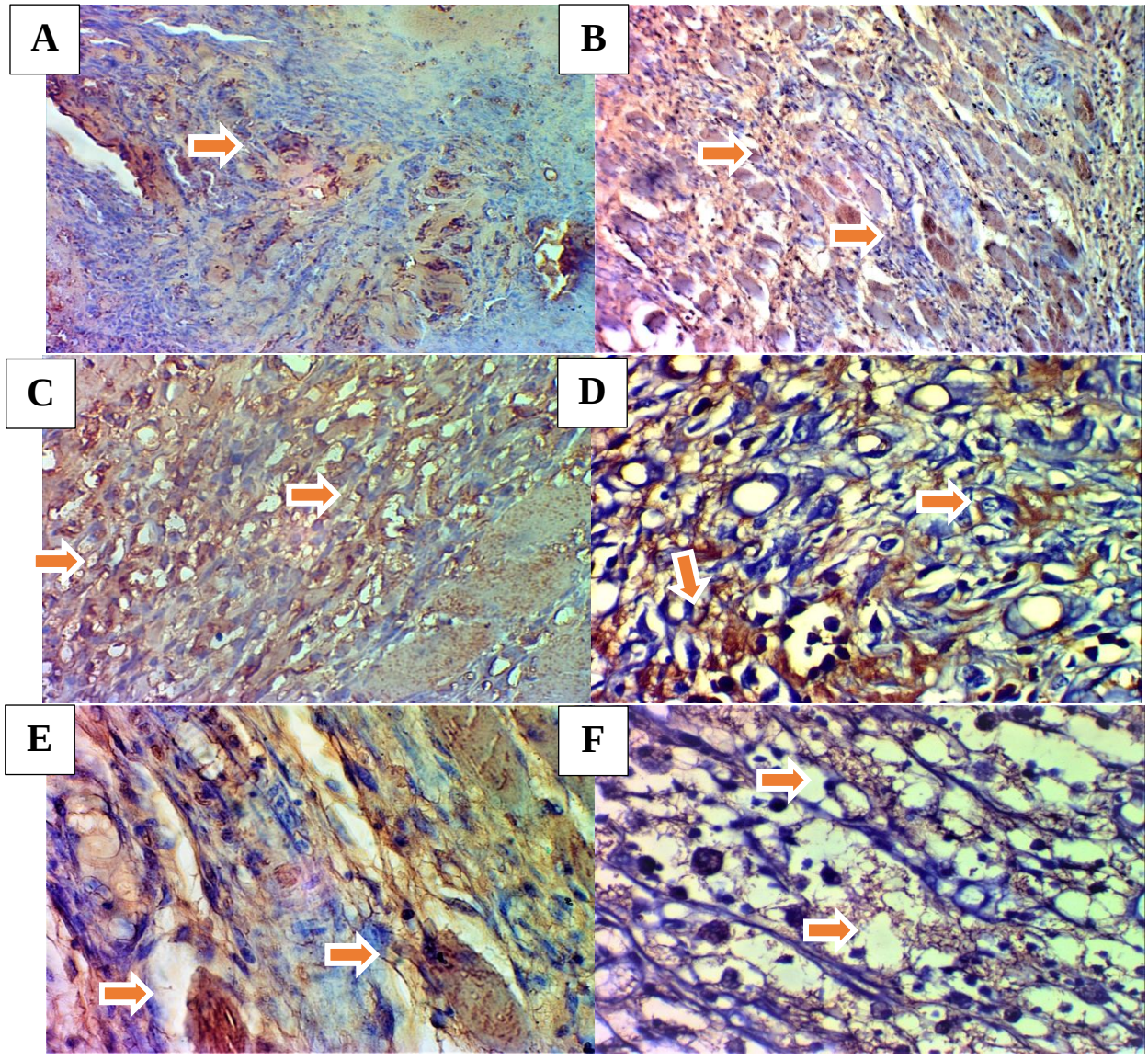

Figure 3: Photomicrograph of FGF2 expression of FD and D groups.

A&B/ The overexpression of FGF2 in group FD rat (arrows) was observed in all areas of dermis layer of wound area. **C/** the expression of FGF 2 (arrows) in group D rat was low compared with the FGF2 expression in group FD rat. **D&E/** The overexpression of FGF2 in group FD rat (arrows) was observed in all areas of dermis layer of wound area. **F/** the expression of FGF 2 (arrows) in group D rat was low compared with the FGF2 expression in group FD rat, also

the low density of collagen fiber was observed in affected area .IHC: DAB and Mayer's hematoxylin. A, C and D: x100 and B, E and F: x400.

Conclusion

The fenugreek oil showed the ability to decreased the expression of MMP2 and stimulate high expression of FGF2 in diabetic rats which led to increase the new fibrous tissue formation in diabetic rats compared with non-treated diabetic rats.

Acknowledgement

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Bioethics committee acceptance

The present study experimental design was accepted by Bioethics committee of university of Kufa under statement no. 15776 in 14/December/ 2020.

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