



Quercetin and low level laser therapy promote wound healing process in diabetic rats *via* structural reorganization and modulatory effects on inflammation and oxidative stress



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ABSTRACT

This study aimed to evaluate the effect of quercetin and the photo-stimulatory effect of low energy 632.8 nm laser irradiation on excisional wound healing in non-diabetic and diabetic rats. Streptozotocin (45 mg/kg body weight) was intraperitoneally applied for diabetes induction. A full-thickness skin wound ($2 \times 2 \text{ cm}^2$) was aseptically created with a scalpel in non-diabetic and diabetic rats on the shaved back of the animals. The wounded non-diabetic and diabetic rats were treated every other day with quercetin by oral gavage at dose 25 mg/kg body weight and/or with low level laser therapy (LLLT) for 14 days. The wound closure percent calculated during the course of the experiment at days 1, 7 and 14 was remarkably increased as a result of treatment of non-diabetic and diabetic wounded rats with quercetin and LLLT; the treatment with both was the most potent. The elevated blood glucose and the lowered serum insulin levels were significantly improved in diabetic wounded rats treated with quercetin and LLLT as compared to the diabetic wounded control. The histological findings indicated that the wounded skin showed a marked increase in collagen fibers which become well oriented in sub-epidermal tissue, intact epidermis and presence of hyperplasia covering well-developed granulation tissue in the wounded rats treated with quercetin and LLLT as compared to the corresponding wounded control. The elevated levels of serum pro-inflammatory cytokines, IL-1 β and TNF- α , as well as PGE-2 and LTB-4 were decreased in non-diabetic and diabetic wounded rats with quercetin and LLLT while the lowered level of serum anti-inflammatory cytokine, IL-10, was increased. The augmented oxidative stress represented by increased serum lipid peroxides level was decreased and the serum level of non-enzymatic anti-oxidant glutathione was increased as a result of treatment with quercetin and LLLT. Thus, it can be suggested that the improvements in glycemic state, cytokines involved in inflammation and antioxidant defense system as well as structural reorganization after treatment with quercetin and LLLT may play pivotal roles in promoting the wound healing process. The study also concluded that the treatment with quercetin in association with LLLT was better in improving wound healing in non-diabetic and diabetic rats than the use of either of each.

1. Introduction

Wounds are major cause of physical disabilities [1]. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide [2]. A wound which is disturbed state of tissue is caused by chemical, physical, microbial or immunological insults or typically associated with loss function [3]. According to the wound healing society, wounds are physical injuries that results in an opening or break of the

skin that cause disturbance in the normal skin function and anatomy [4].

Diabetes mellitus (DM) is a complex metabolic chronic disorder involving many organs and tissues and continue to devastate the lives of affected individuals [5]. Impaired wound healing is one of complications of DM and it is a serious problem in clinical practice [6]. It was estimated that 15% of individuals with DM will develop foot ulceration and wounds, and 3% will require lower-extremity amputation [7]. In

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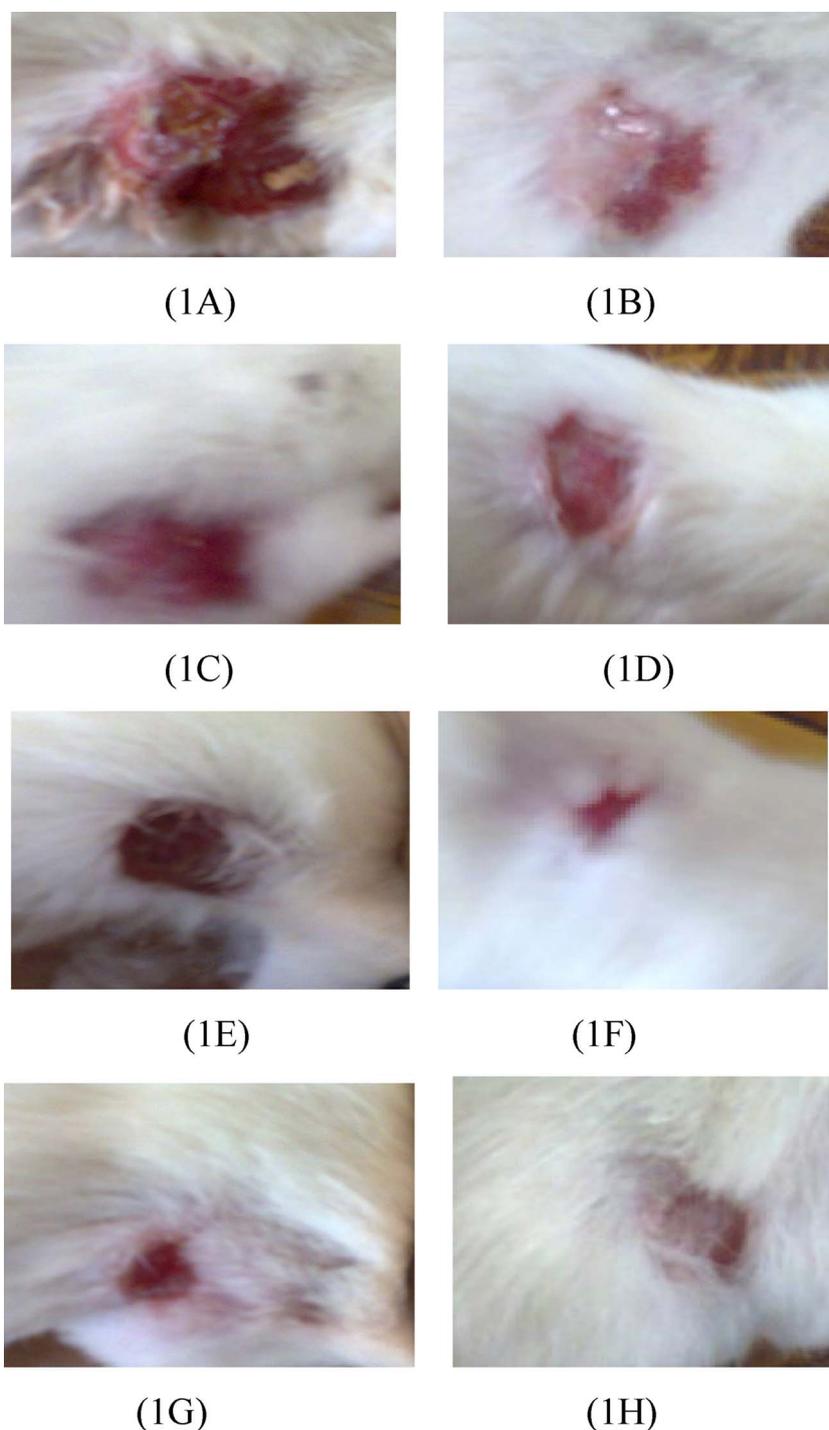


Fig. 1. Photographs showing wound healing and wound closure in untreated and treated non-diabetic wounded rats. 1A: wound at the 7th day in untreated non-diabetic rat. 1B: wound at the 14th day in untreated non-diabetic rat. 1C: wound at the 7th day in non-diabetic wounded rat treated with quercetin. 1D: wound at the 14th day in non-diabetic wounded rat treated with quercetin. 1E: wound at the 7th day in non-diabetic wounded rat treated with LLLT. 1F: wound at the 14th day in non-diabetic wounded rat treated with LLLT. 1G: wound at the 7th day in non-diabetic wounded rat treated with quercetin and LLLT. 1H: wound at the 14th day in non-diabetic wounded rat treated with quercetin and LLLT.

addition, an increased incidence of wound complications in surgical patients with DM increases the general surgical risks due to the metabolic abnormalities associated with DM [8]. In our previous publication, it was demonstrated that although DM slows wound healing, daily administration of natural product, camel whey protein, after wounding hastens wound closure in both diabetic and non-diabetic rats [9].

It has been found that more than 80% of the world population is dependent on the drugs from natural origin for the treatment of skin related problems [10]. Many of the synthetic drugs are associated with problems like drug resistance, allergy and other side effects making the

scientists to seek alternative drugs [11]. Natural healing process of wound management involves disinfection, debridement and providing a moist environment [9], thereby hastening the healing process. Plant based therapy not only accelerates wound healing process but also maintains the aesthetics in a natural way [12]. More than 70% of wound healing pharmaceutical products are derived from plants, 20% are mineral based and the remaining are animal-derived products [13]. Plant-derived materials are used as first aid-antiseptic coagulants and wound wash [2]. In recent times, focus on plant researches has increased all over the world and large body of evidence has collected to

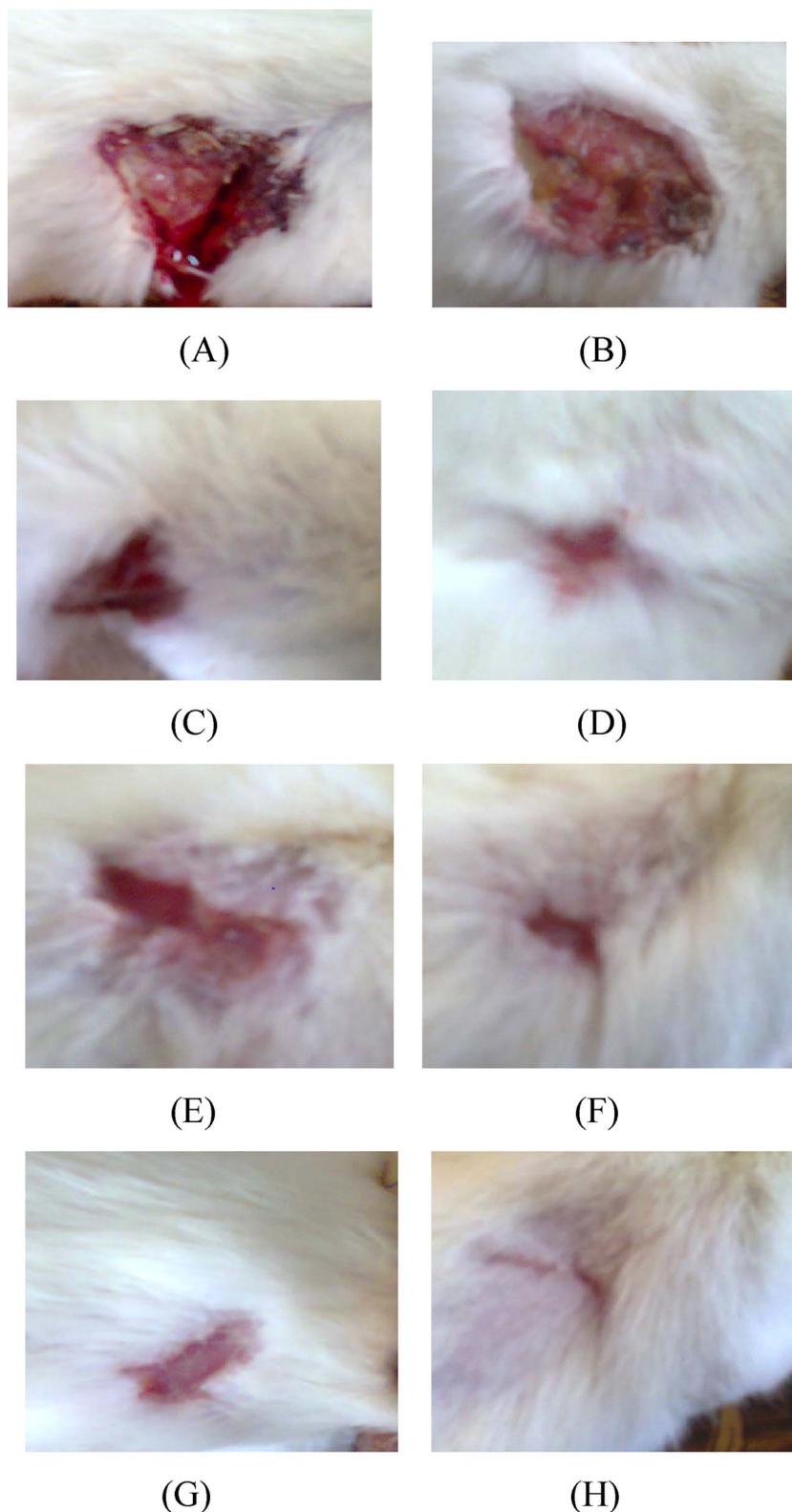


Fig. 2. Photographs showing wound healing and wound closure in untreated and treated wounded diabetic rats. 2A: wound at the 7th day in untreated diabetic rat. 2B: wound at the 14th day in untreated diabetic rat. 2C: wound at the 7th day in diabetic rat treated with quercetin. 2D: wound at the 14th day in diabetic rat treated with LLLT. 2E: wound at the 7th day in diabetic rat treated with LLLT. 2F: wound at the 14th day in diabetic rat treated with LLLT. 2G: wound at the 7th day in diabetic rat treated with quercetin and LLLT. 2H: wound at the 14th day in diabetic rat treated with quercetin and LLLT.

show immense potential of medicinal plants used in various traditional systems [14]. According to Biswas and Mukarje in 2003, more than 13,000 plants have been studied [14]. These reviews and researches probably provide findings for wound healing activity of some medicinal

plants and their ingredient especially those which have potent anti-oxidant and anti-inflammatory properties. Quercetin, one plant ingredient which was described by some previous publications as the most important flavonoid, has been reported to have potent anti-

Table 1
Effect of treatment with quercetin and low level laser therapy (LLLT) on wound closure percent in non-diabetic and diabetic wounded rats.

Groups	Wound closure percent		
	1 day	7 days	14 days
Non-diabetic wounded	0.0 ± 0.0 ^l	35.0 ± 0.9 ^l	85.0 ± 0.9 ^c
Non-diabetic wounded treated with quercetin	0.0 ± 0.0 ^l	63.3 ± 2.8 ^g	83.3 ± 1.0 ^c
Non-diabetic wounded treated with LLLT	0.0 ± 0.0 ^l	74.9 ± 1.0 ^e	91.6 ± 1.0 ^b
Non-diabetic wounded treated with quercetin and LLLT	3.3 ± 1.0 ^k	79.2 ± 0.5 ^d	100.0 ± 0.0 ^a
Diabetic wounded	0.0 ± 0.0 ^l	18.3 ± 1.1 ^l	36.7 ± 1.0 ^l
Diabetic wounded treated with quercetin	0.0 ± 0.0 ^l	51.7 ± 2.1 ^h	83.3 ± 1.1 ^c
Diabetic wounded treated with LLLT	0.0 ± 0.0 ^l	68.3 ± 1.0 ^f	76.7 ± 1.1 ^{de}
Diabetic wounded treated with quercetin and LLLT	4.2 ± 0.0 ^k	79.2 ± 0.6 ^d	98.3 ± 1.0 ^a

Data are expressed as mean ± standard error. Number of animal in each group is six. For each parameter, the means which share the same superscript symbol(s) are not significantly different.

F-probability: $p < 0.001$; LSD at 5%: 3.009; LSD at 1%: 4.052.

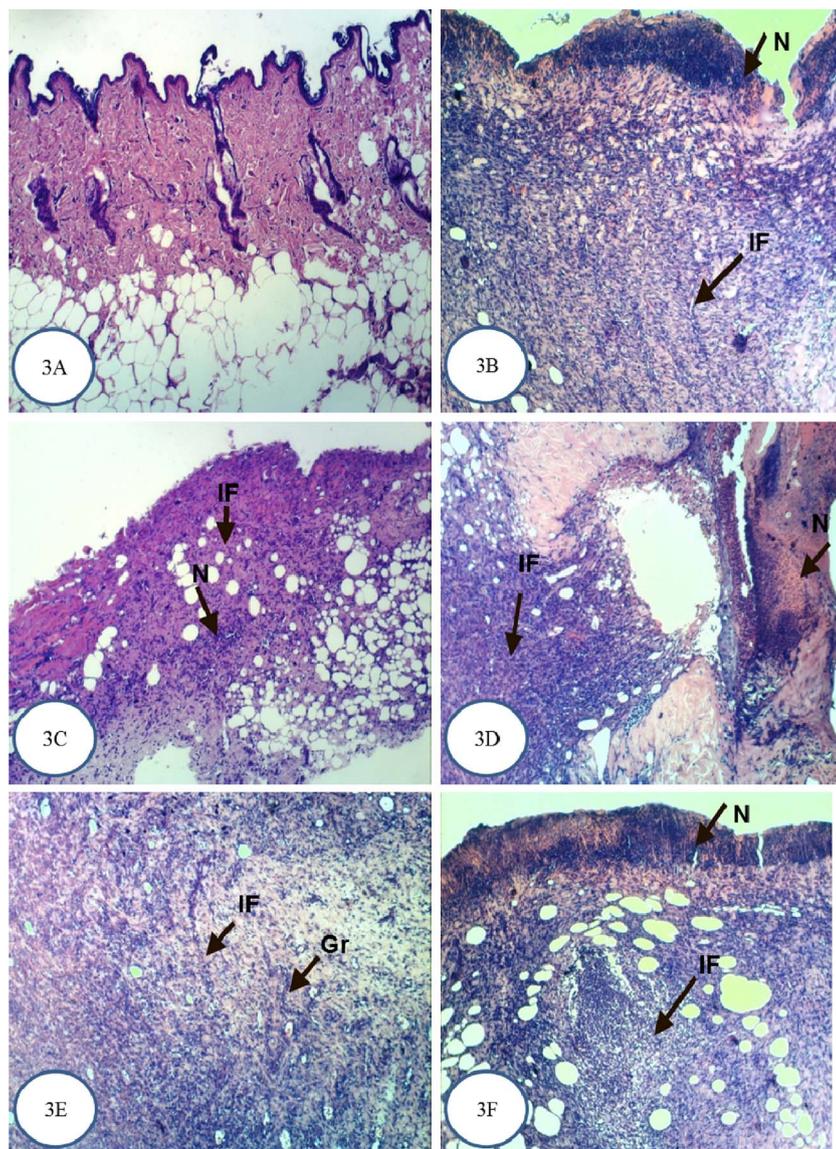


Fig. 3. Photomicrographs of vertical section (v. s.) of skin of non-diabetic un-injured (3A) and wounded (3B-3F) rats at day 7 after wounding. 3A: Photomicrograph of a v. s. in skin of non-diabetic non-wounded control rat showing no histopathological changes. 3B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing marked necrosis (N) and massive inflammatory cells infiltration (IF). 3C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing marked necrosis (N) and massive inflammatory cells infiltration (IF). 3D & 3E: Photomicrographs of v. s. in skin of non-diabetic wounded rats treated with LLLT showing marked necrosis (N), granulation tissue formation (Gr) and inflammatory cells infiltration (IF). 3F: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin and LLLT showing marked necrosis (N) in the thickened epidermis and massive inflammatory cells infiltration (IF) in the dermis. (H & E X 100).

inflammatory and antioxidant activities [15].

Laser light has the unique properties of monochromaticity (a single wavelength), collimation (travels in a single direction without divergence) and coherence (with all waves in phase) [16]. These properties are what allow laser light to non-invasively penetrate the skin surface [16,17]. Therapeutic lasers are athermic with no appreciable heat transfer ($< 0.65^{\circ}\text{C}$); so, the photonic energy is directly transferred to the target cells and thermal damage is avoided [16,17]. Therapeutic lasers use monochromatic light in the range from 630 to 905 nm, and is also known as the “therapeutic window” [18]. Aboud et al. [19] suggested that LLLT and quercetin treatment facilitates the tissue repair process by accelerating collagen production in diabetic wound healing.

In conductance with the previous publications, this study was designed to assess the efficacy of quercetin treatment and low level laser therapy (LLLT) on enhancing the wound healing and recovery in non-diabetic and streptozotocin (STZ)-induced diabetic rats and to explore the roles of anti-inflammatory effects and antioxidant defense system in healing process.

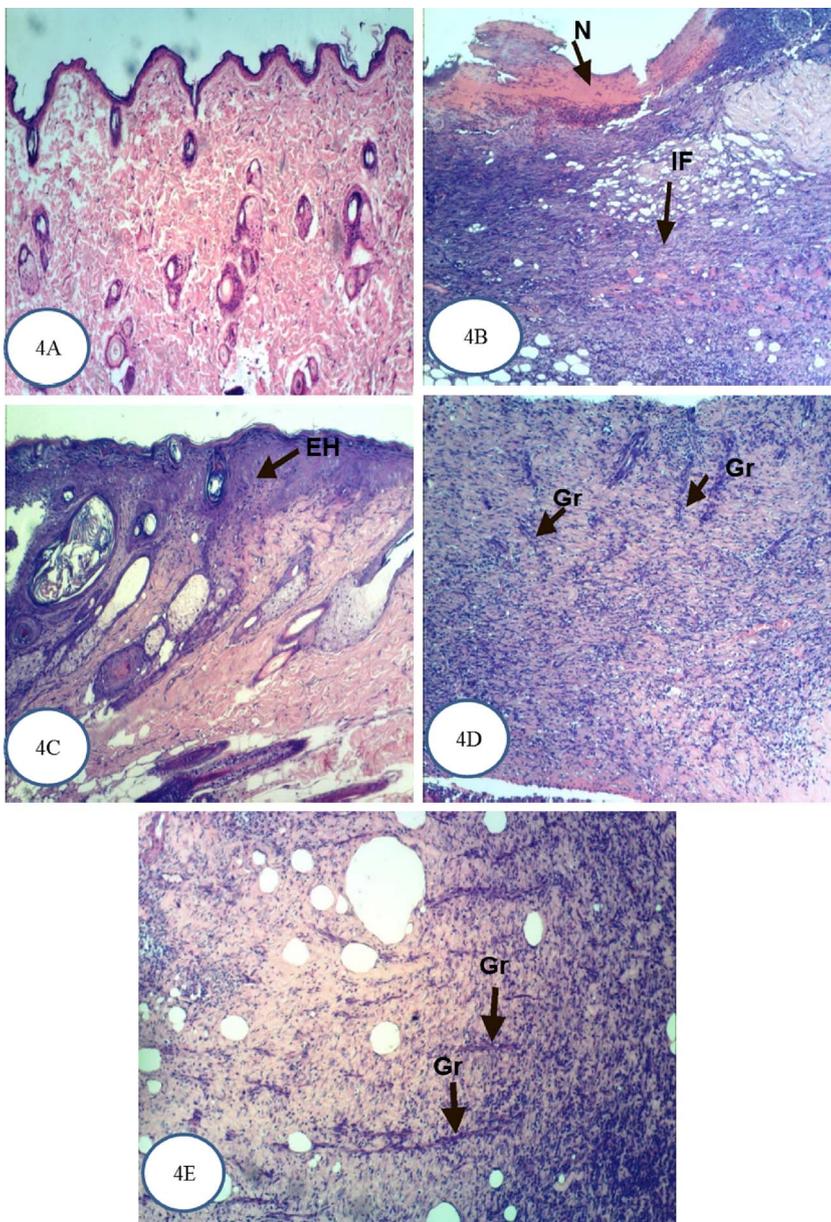


Fig. 4. Photomicrographs of v. s. of skin of non-diabetic un-injured (4A) and wounded (4B–4E) rats at day 14 after wounding. 4A: Photomicrograph of a v. s. in skin of non-diabetic non-wounded control rat showing no histopathological changes. 4B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing marked necrosis (N) and massive inflammatory cells infiltration (IF) with granulation tissue formation. 4C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing epidermal hyperplasia. 4D: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with LLLT showing well oriented granulation tissue formation (Gr). 4E: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated quercetin and LLLT showing well oriented granulation tissue formation (Gr). (H & E X 100).

2. Materials and methods

2.1. Experimental animals

A total of one hundred male albino rats weighting 100–120 g obtained from National Research Centre, Dokki, Giza, Egypt, were used in the present investigation. The rats were maintained at a temperature of 22–25 °C with a 12-hours light/dark cycle and were allowed free access to water and standard pelleted diet *ad libitum* in the Animal House of Zoology Department, Faculty of Science, Beni-Suef University, Egypt. All animals' procedures are in accordance with the guidelines of Experimental Animal Ethics Committee, Faculty of Science, Beni-Suef University, Egypt. All efforts were done to minimize the suffering of animals. The Ethical Approval Number is BSU/FS/2014/6.

2.2. Chemicals

STZ and quercetin were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other used chemicals were of analytical grade.

2.3. Induction of DM

STZ was dissolved in cold citrate buffer (pH 4.5) and was freshly prepared for immediate use within few minutes. STZ was intraperitoneally injected as a single dose of 45 mg/kg body weight (b. wt) [19,20] to rats deprived of food and water for sixteen hours (hrs). The dose of STZ was administered at a volume 1 ml/kg b. wt rats. Ten days after STZ injection, rats were deprived of food overnight (10–12 h) and then were orally administered glucose at dose level of 3 g/kg b. wt after 2 h of oral glucose gavage (3 g/kg b. wt). Blood drop were obtained from lateral tail vein of each rat and blood glucose concentration was measured by Glucometer (Model Bionime Rightest GM 100). The rats that have blood glucose concentration ranged from 180 to 300 mg/dl were considered as mild diabetic rats and were included in the experiment while others were excluded.

2.4. Wounding operation

Rats were anaesthetized by intraperitoneal administration of ketamine at dose level of 60 mg/kg b. wt. [21]. The dorsal fur of the

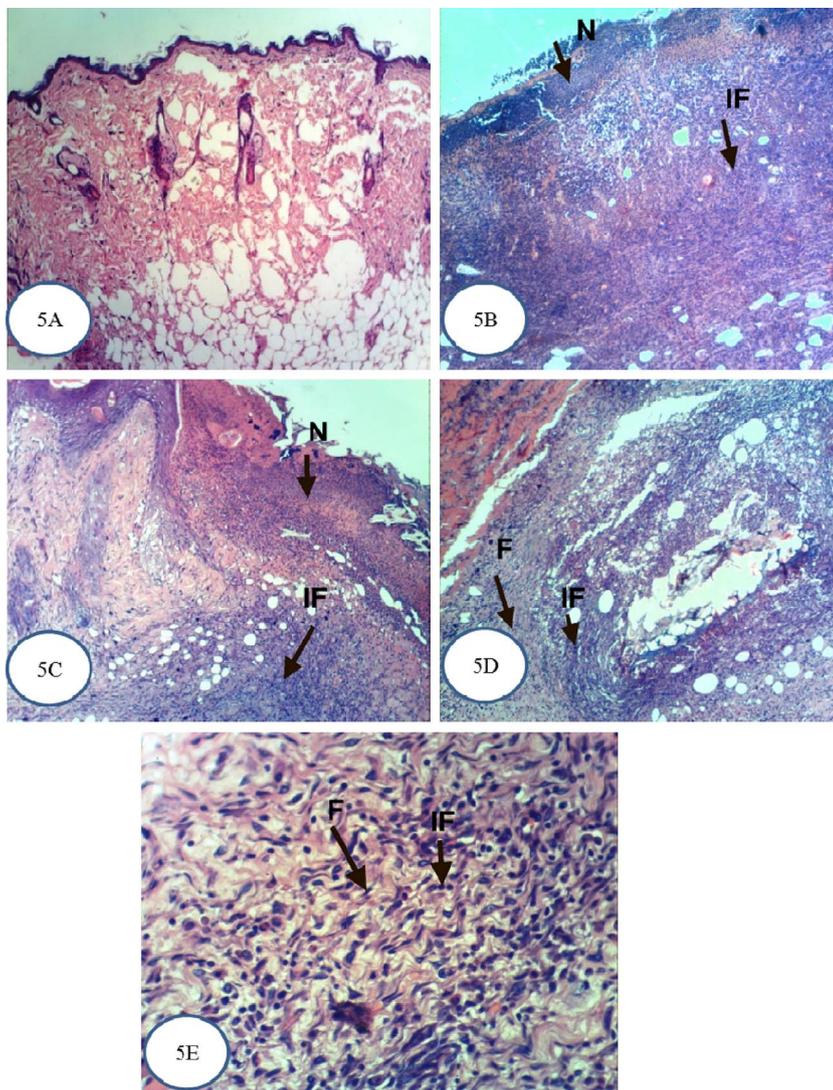


Fig. 5. Photomicrographs of v. s. of skin of diabetic un-injured (5A) and diabetic wounded (5B-5E) rats at day 7 after wounding. 5A: Photomicrograph of a v. s. in skin of diabetic non-wounded control rat showing normal structure of epidermis and dermis. 5B: Light micrograph of a v. s. in skin of diabetic wounded rat showing necrosis (N) associated with massive inflammatory cells infiltration (IF). 5C: Photomicrograph of a v. s. in skin of diabetic wounded rat treated with quercetin showing necrosis (N) associated with massive inflammatory cells infiltration (IF). 5D: Photomicrograph of a v. s. in skin of diabetic wounded rat treated with LLLT showing dermal inflammatory cells infiltration (IF) in association with fibroblasts proliferation (F) (H & E; 100X). 5E: Photomicrograph of a v. s. in skin of diabetic wounded rat treated with quercetin and LLLT showing dermal inflammatory cells (IF) infiltration associated with fibroblasts proliferation (F). (H & E, X 400).

anaesthetized rats was shaved with an electric clipper, and the area of the wound was outlined on the back of the rats with a marker pen. An excision wound of size 2 cm² was made by cutting out a 2 × 2 cm piece of skin from the shaved area. The wounds were of the same full thickness type extending up to the subcutaneous tissue [19,22].

2.5. Dose preparation of quercetin

Quercetin was administered to rats at dose level of 25 mg/kg b. wt every other day for two weeks [19]. For dose preparation, 25 mg of quercetin was dissolved in 5 ml of 1% carboxymethylcellulose (CMC) that was administered to kg of rats [19].

2.6. Low level laser therapy (LLLT)

Laser used for therapy in this study was He-Ne laser (NEC, Japan). Applied laser has a wavelength of 632.8 nm, spot size of the laser beam of 1 cm² with incident dose of 6.36 Joules/cm², power density of 3 mW/cm², beam cross-section of 0.3 cm², and application time of 10 min [19].

2.7. Animal grouping

After induction of diabetes mellitus (10 days post-STZ injection) and wounding, the considered rats were allocated into the following groups

(ten animals for each).

Group 1: Rats of this group were non-diabetic non-wounded.

Group 2: Rats included in this group were non-diabetic wounded.

Group 3: Rats of this group were non-diabetic wounded and were administered quercetin at dose level of 25 mg/kg b. wt every other day by oral gavage for 14 days.

Group 4: Rats of this group were non-diabetic wounded and were subjected to LLLT every other day for 14 days.

Group 5: Rats included this group were non-diabetic wounded and were also treated with quercetin by oral gavage and subjected to LLLT every other day for 14 days.

Group 6: Rats included in this group were diabetic non-wounded control.

Group 7: Rats of this group were diabetic wounded.

Group 8: Rats included in this group were diabetic wounded and were administered quercetin at dose level of 25 mg/kg b. wt every other day by oral gavage for 14 days.

Group 9: Rats included in this group were diabetic wounded rats subjected every other day to LLLT for 14 days.

Group 10: Rats of this group were diabetic wounded and were treated with quercetin by oral gavage and subjected to LLLT every other day for 14 days.

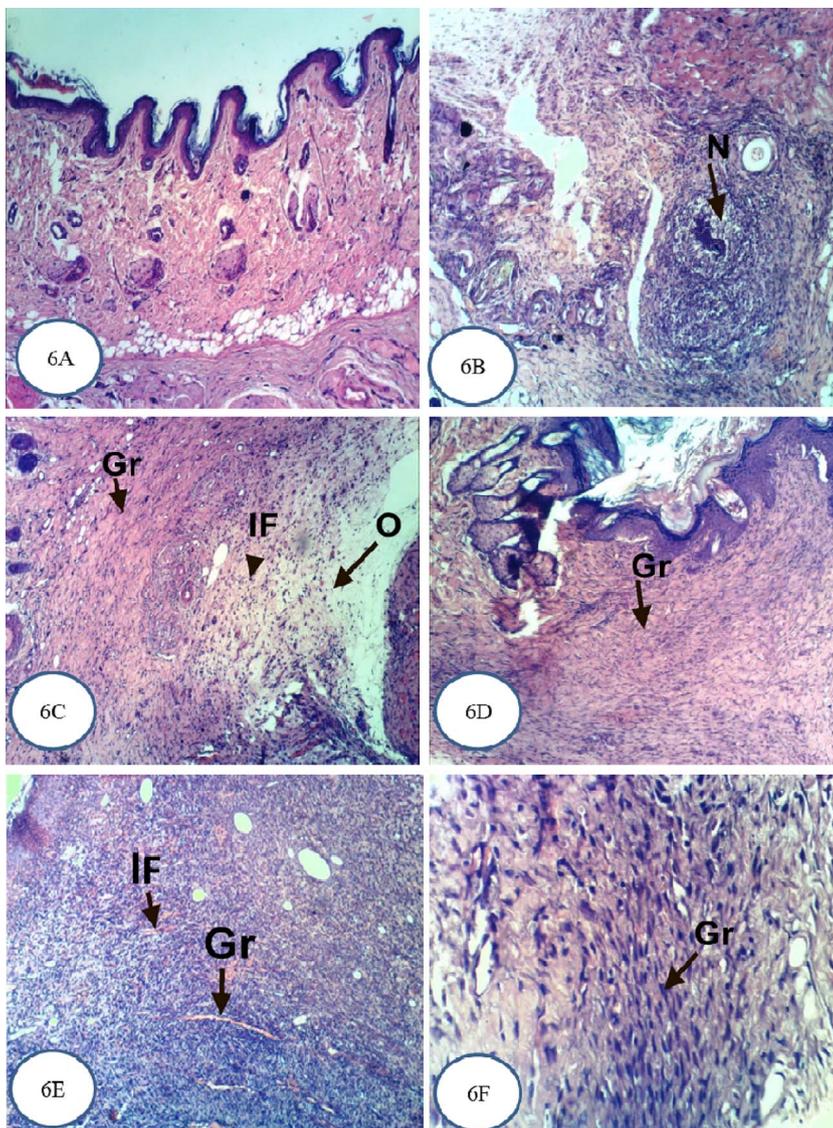


Fig. 6. Photomicrographs of v. s. of skin of diabetic un-injured (6A) and diabetic wounded (6B–6F) rats at day 14 after wounding. 6A: Photomicrograph of a v. s. in skin of diabetic non-wounded control rat showing normal structure of epidermis and dermis. 6B: Photomicrograph of a v. s. in skin of diabetic wounded rat showing necrosis associated with massive inflammatory cells infiltration (N). 6C: Photomicrograph of a v. s. in skin of diabetic wounded rat treated with quercetin showing granulation tissue formation (Gr), edema (O) and inflammatory cells infiltration (IF). 6D: Photomicrograph of a v. s. in skin of diabetic wounded rat treated with LLLT showing dermal granulation tissue formation (Gr) (H&E, X100). 6E & 6F: Photomicrographs of v. s. in skin of diabetic wounded rats treated with quercetin and LLLT showing granular tissue formation, inflammatory cells infiltration and collagen fibers production. (H&E, X 100 & 400X).

2.8. Collection of the blood samples

After 7 and 14 days of treatment period, blood samples were collected from jugular vein of fasted rats under diethyl ether anesthesia, left to coagulate at room temperature and then centrifuged at 3000 rpm for 15 min. The supernatant sera were aspirated and fractioned into three Eppendorf tubes.

2.9. Measurement of wound diameter and closure

The wound diameter was measured on days 1, 7 and 14 after incision and wound closure percent was calculated using the following equation: wound closure rate on day X (%) = $[(\text{wound diameter on day 0} - \text{wound diameter on day X}) / (\text{wound diameter on day 0})] \times 100$ [19,21].

2.10. Estimation of blood glucose and serum insulin levels

The blood glucose level was determined by Glucometer (Model Bionime Rightest GM 100). Serum insulin level was detected by Sandwich ELISA according manufacturer's instructions using kits purchased from Linco Research, USA.

2.11. Estimation of serum cytokine levels

Serum IL-1 β , TNF- α , PGE-2, LTB-4 and IL-10 levels were assayed by ELISA kit obtained from Scientific Group S.A, BD550788 according to the manufacture's instruction.

2.12. Estimation of serum lipid peroxides (LPO) and glutathione (GSH) levels

Serum lipid peroxides (LPO) and glutathione (GSH) levels were determined according to the procedures of Preuss et al. [23] and Beutler et al. [24] respectively by using reagents prepared in laboratory.

2.13. Histopathological examination

After sacrifice and decapitation, rats were dissected and skin in the regions of wounds was rapidly excised and fixed in 10% neutral buffered formalin for 48 h. The fixed skin of each rat was then processed for wax blocking and sectioning. The sections were taken at 5 μ m thickness using microtome, processed in xylene alcohol-series and stained with alum hematoxylin and eosin (H&E) [25]. The sections were examined microscopically for the evaluation of histopathological changes.

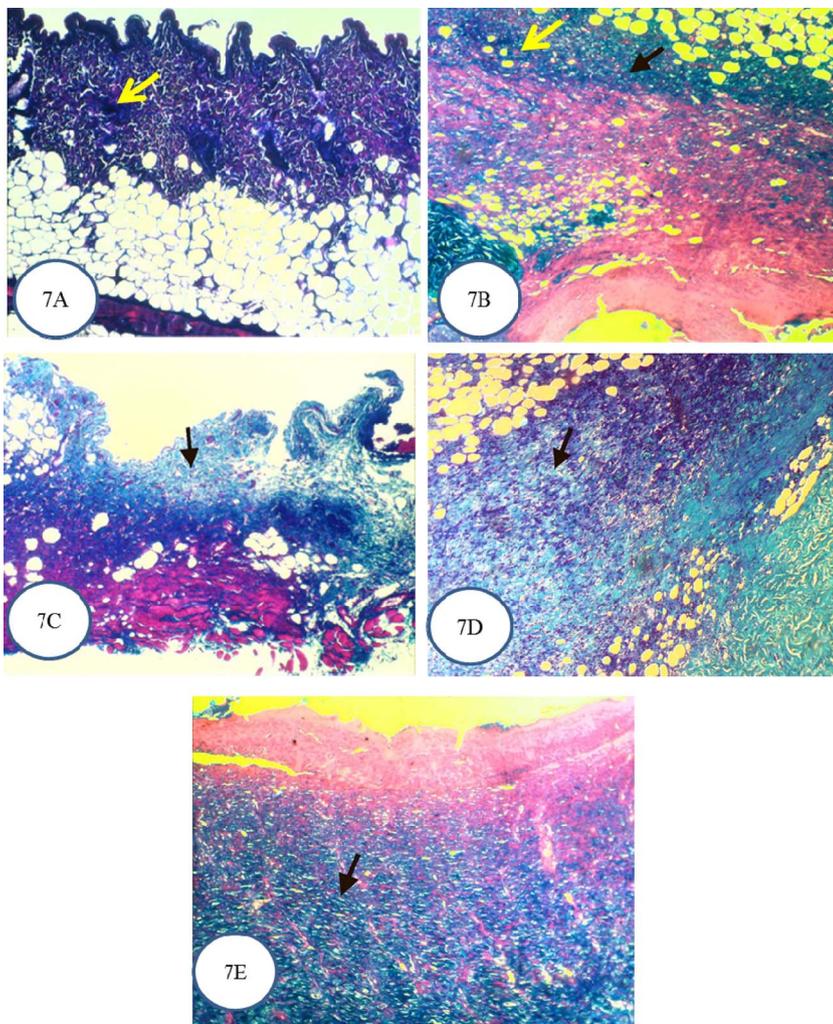


Fig. 7. Photomicrographs of Masson's Trichrome stained sections of skin of non-diabetic un-injured rat (7A) and wounded rats (7B–7E) at day 7 after wounding showing dermal collagen fibers. 7A: Photomicrograph of a v. s. in skin of non-diabetic non wounded control rat showing normal collagen fibers content in the dermis. 7B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing haphazardly arranged few collagen fibers in the dermis. 7C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing few collagen fibers in the dermis. 7D: Photomicrograph of v. s. in skin of non-diabetic wounded rat treated with LLLT showing few collagen fibers in the dermis. 7E: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin and LLLT showing few collagen fibers in the dermis. (Masson's Trichrome stain X 100).

2.14. Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) (PC-STAT, University of Georgia, 1985) [26] followed by the LSD test to compare various groups with each other. The results were expressed as mean \pm SE, and values of $P > 0.05$ were not considered significantly different, whereas values of $P < 0.05$ were considered significant.

3. Results

3.1. Wound closure

The wound closure in non-diabetic wounded rats was measured at the 1st, 7th and 14th days after injury and was depicted in Figs. 1 & 2 and Table 1. The wound closure was remarkably improved as a result of treatment of non-diabetic wounded and diabetic wounded rats with quercetin, LLLT or quercetin and LLLT; treating with both was the most potent.

In untreated non-diabetic wounded rats, the wound closure percent of the wound reached 0% at day 1 after injury, 63.3% at day 7 after injury and 83.3% at day 14 after injury. This closure percent was greatly accelerated after quercetin treatment and LLLT as compared to the control wounded rats where the percent of wound closure was increased to 3.3% at day 1, 79.2% at day 7 and 100% at day 14 after injury. The wound site was covered by epidermis recording wound closure percent of 100% as a result of treatment with both quercetin

and LLLT at the end of the experiment. While the effect of quercetin treatment was significant ($p < 0.01$; LSD) only at day 7, the effect of LLLT was significant ($p < 0.01$) at days 7 and 14 and the effect of the two treatments together was significant at days 1 ($p < 0.05$), 7 ($p < 0.01$) and 14 ($p < 0.01$).

In untreated diabetic rats, the wound closure was more severely affected than in the untreated non-diabetic wounded rats since the wound closure percent reached 0% at day 1 and increased to 18.3% at day 7 and to 36.7% at day 14 after injury. In diabetic rats treated with quercetin and LLLT, the wound closure percent reached 4.2% at day 1 after injury and increased to 79.2% at day 7 after and to 98.3% at day 14 after injury. Thus, the treatment of non-diabetic or diabetic wounded rats with quercetin and LLLT in combination produced the most potent effect on wound closure percent than the treatment with either quercetin or LLLT. Moreover, statistical analysis by LSD indicated that the treatment with either quercetin or LLLT induced a significant increase ($p < 0.01$; LSD) in wound closure percent at days 7 and 14 while both treatments together induced a significant increase ($p < 0.01$; LSD) at days 1, 7 and 14 after injury. One-way ANOVA revealed that the effect between groups was significant ($p < 0.001$; F-probability) on wound closure percent throughout the experiment.

3.2. Histological effects

The histopathological findings of the skin at region of wounds are depicted in photomicrographs of Figs. 3–6.

The skin of non-diabetic non-wounded control rats at day 7 after

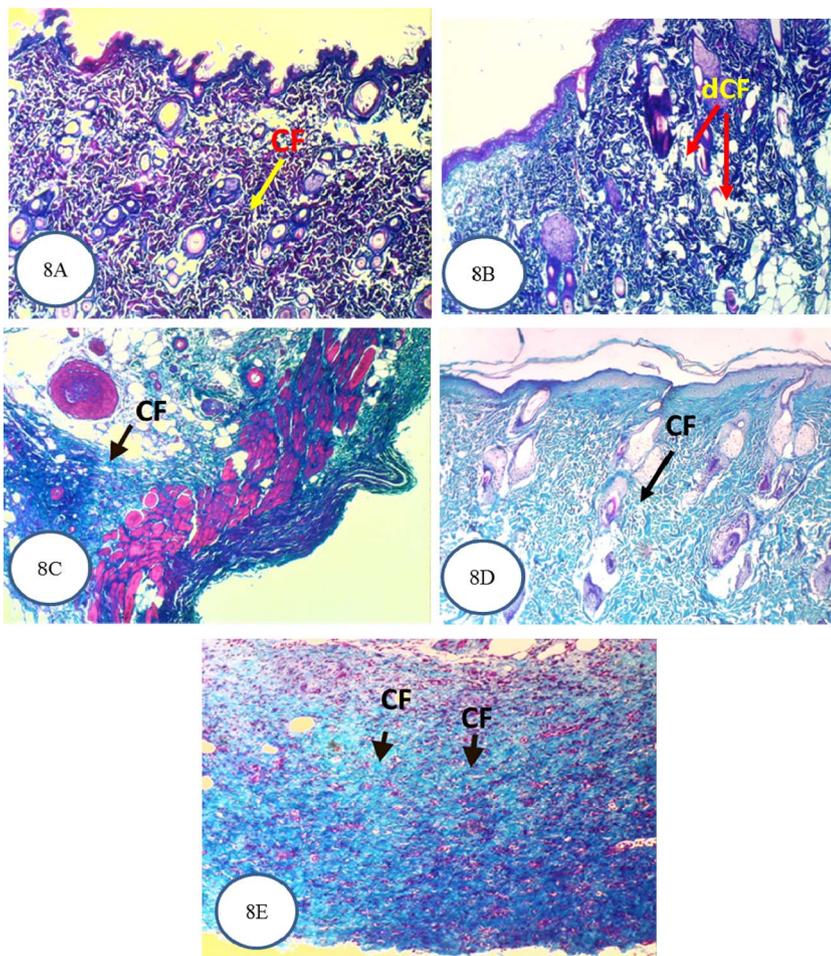


Fig. 8. Photomicrographs of Masson's Trichrome stained sections of skin of non-diabetic un-injured rat (8A) and wounded rats (8B–8E) at day 14 after wounding showing collagen. 8A: Photomicrograph of a v. s. in skin of non-diabetic non-wounded control rat showing normal collagen fibers in the dermis. 8B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing regions devoid of collagen fibers in the dermis. 8C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing non-oriented collagen fibers in the dermis. 8D: Photomicrograph of v. s. in skin of non-diabetic wounded rat treated with LLLT showing normal collagen fibers in the dermis. 8E: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin and LLLT showing normal collagen fibers in the dermis. (Masson's Trichrome stain X 100).

injury showed normal histological structure of epidermis and dermis (Photomicrograph 3A). The skin of non-diabetic wounded rats at day 7 after injury exhibited a marked necrosis and a massive inflammatory cells infiltration (Photomicrograph 3B). The skin of non-diabetic wounded rats treated with quercetin at day 7 after injury still exhibited remarkable deleterious histological lesions marked by moderate necrosis and inflammatory cells infiltration (Photomicrograph 3C). As a result LLL irradiation, the skin of non-diabetic wounded rats treated with LLLT after one week of injury showed marked granulation tissue formation but still revealed marked necrosis and inflammatory cells infiltration (Photomicrographs 3D and 3E). The skin of non-diabetic wounded rats treated with both quercetin and LLLT at day 7 after injury showed marked necrosis and massive inflammatory cells infiltration and the epidermis appeared more thickened as compared to that of non-diabetic un-injured skin (Photomicrograph 3F).

The skin of non-diabetic un-injured rats (Photomicrograph 4A) and non-diabetic wounded ones (Photomicrographs 4B–4E) at day 14 after wounding was also investigated. The skin of non-diabetic non-wounded control rats depicted no histopathological changes with normal architecture of epidermis and dermis (Photomicrograph 4A). On the other hand, the skin of non-diabetic wounded rats exhibited marked necrosis and massive inflammatory cells infiltration in association with granulation tissue formation (Photomicrograph 4B). The skin of non-diabetic wounded rats treated with quercetin showed no or mild dermal inflammatory cells infiltration and epidermal hyperplasia (Photomicrograph 4C). The LLLT or the treatment with quercetin in association with LLLT of non-diabetic rats resulted in potential improvements and well oriented granulation tissue formation (Photomicrographs 4D and 4E).

Similar to non-diabetic non-wounded rats, the skin of diabetic non-

wounded control rat at day 7 exhibited normal structure of epidermis and dermis (Photomicrograph 5A). On the other hand, the skin of diabetic wounded rat showed necrosis associated with massive inflammatory cells infiltration (Photomicrograph 5B). The skin of diabetic wounded rats treated with quercetin exhibited necrosis associated with massive inflammatory cells infiltration (Photomicrograph 5C). The skin of diabetic wounded rats treated with LLLT (Photomicrograph 5D) or treated with quercetin and LLLT (Photomicrograph 5E) showed dermal inflammatory cells infiltration in association with fibroblasts proliferation.

The skin of diabetic un-injured and diabetic wounded rats at day 14 after wounding is illustrated in Fig. 6. The skin of diabetic non-wounded control rat showed normal structure of epidermis and dermis (Photomicrograph 6A). The skin of diabetic wounded rat showed necrosis associated with massive inflammatory cells infiltration (Photomicrograph 6B). The diabetic wounded rats treated with quercetin exhibited marked amelioration detected by granulation tissue formation; however, edema and inflammatory cells infiltration were found (Photomicrograph 6C). The exposure of skin of diabetic wounded rats to LLLT enhanced remarkable recovery of wound healing marked by dermal granulation tissue formation (Photomicrograph 6D). The skin of diabetic wounded rats treated with quercetin and LLLT also revealed granular tissue formation and collagen fibers production, but inflammatory cells are still found (Photomicrographs 6E and 6F).

3.3. Effect on the skin collagen fibers

The photomicrographs of Masson's Trichrome stained sections depicting density and distribution of collagen fibers in the dermis of the skin are shown in Figs. 7–10.

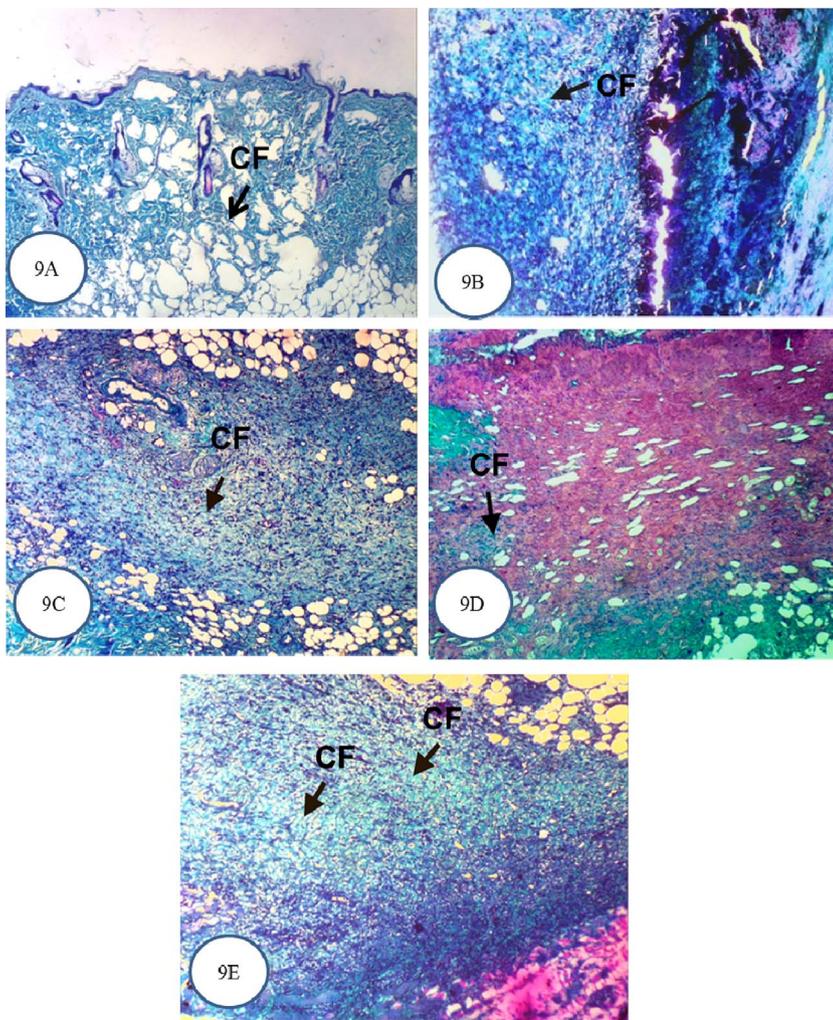


Fig. 9. Photomicrographs of Masson's Trichrome stained sections of skin of diabetic non-wounded control rat (9A) and diabetic wounded rats (9B–9E) at day 7 after wounding showing dermal collagen fibers. 9A: Photomicrograph of a v. s. in skin of diabetic non-wounded control rat showing normal collagen fibers content in the dermis. 9B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing non well-oriented collagen fibers in the dermis. 9C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing randomly arranged collagen fibers in the dermis. 9D: Photomicrograph of v. s. in skin of non-diabetic wounded rat treated with LLLT showing few collagen fibers in the dermis. 9E: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin and LLLT showing not well-oriented collagen fibers with increased density in the dermis. (Masson's Trichrome stain X 100).

Fig. 7 represented photomicrographs of Masson's Trichrome stained sections of the skin of non-diabetic non-wounded rats (Photomicrograph 7A) and wounded rats (photomicrographs 7B–7E) at day 7 after wounding showing collagen fibers in the dermis. The skin of non-diabetic non-wounded control rats depicted normal collagen fibers content in the dermis. The non-diabetic wounded rat exhibited haphazardly arranged few collagen fibers in the dermis. On the other hand, the non-diabetic wounded rats treated with quercetin (photomicrograph 7C), LLLT (photomicrograph 7D) or quercetin and LLLT (photomicrograph 7E) exhibited few collagen fibers in the dermis of the skin in the regions of wounds.

Fig. 8 showed photomicrographs of Masson's Trichrome stained sections of the skin of non-diabetic non-wounded rats (Photomicrograph 8A) and non-diabetic wounded rats (Photomicrographs 8B–8E) at day 14 after wounding depicting dermal collagen fibers. The skin of non-diabetic non-wounded control rats demonstrated normal collagen fibers density and organization in the dermis (Photomicrograph 8A). The skin of non-diabetic wounded rats at day 14 after injury showed regions devoid of collagen fibers in the dermis (Photomicrograph 8B). On the other hand, the skin of non-diabetic wounded rats treated with quercetin exhibited non-oriented collagen fibers in the dermis (Photomicrograph 8C) while the non-diabetic wounded rats treated with LLLT (Photomicrograph 8D) or with quercetin and LLLT (Photomicrograph 8E) manifested normal collagen fibers in the dermis.

Fig. 9 depicted photomicrographs of Masson's Trichrome stained sections of the skin of diabetic non-wounded control rats (Photomicrograph 9A) and diabetic wounded rats (photomicrographs 9B–9E) at day 7 after wounding showing dermal collagen fibers. The skin of

diabetic non-wounded control rats demonstrated normal collagen fibers content in the dermis (Photomicrograph 9A) whereas the skin of non-diabetic wounded rats showed non well-oriented collagen fibers in the dermis (Photomicrograph 9B). The skin of non-diabetic wounded rats treated with quercetin depicted randomly arranged dermal collagen fibers (photomicrograph 9C). The skin of non-diabetic wounded rat treated with LLLT demonstrated few collagen fibers in the dermis (Photomicrograph 9D). Moreover, the non-diabetic wounded rats treated with quercetin in association with LLLT showed non well-oriented dense collagen fibers in the dermis (Photomicrograph 9E).

Fig. 10 is composed of photomicrographs of Masson's Trichrome stained sections of the skin of diabetic non-wounded rats (Photomicrograph 10A) and diabetic wounded rats (Photomicrographs 10B–10E) at day 14 after wounding showed collagen orientation and density in the dermis. The skin of diabetic non-wounded control rat depicted well-oriented and homogeneously distributed collagen fibers in the dermis (Photomicrograph 10A) but the diabetic wounded rats exhibited non well-oriented collagen fibers in the dermis (Photomicrograph 10B). The skin of diabetic wounded rat treated with quercetin (Photomicrograph 10C) or LLLT (Photomicrograph 10D) showed randomly oriented and haphazardly arranged collagen fibers in the dermis while the diabetic wounded rat treated with quercetin in association with LLLT explored well oriented dermal collagen fibers.

Overall, the treatment with quercetin in association with LLLT was the most effective in improving the integrity and orientation of collagen fibers in the wounded skin in non-diabetic and diabetic rats.

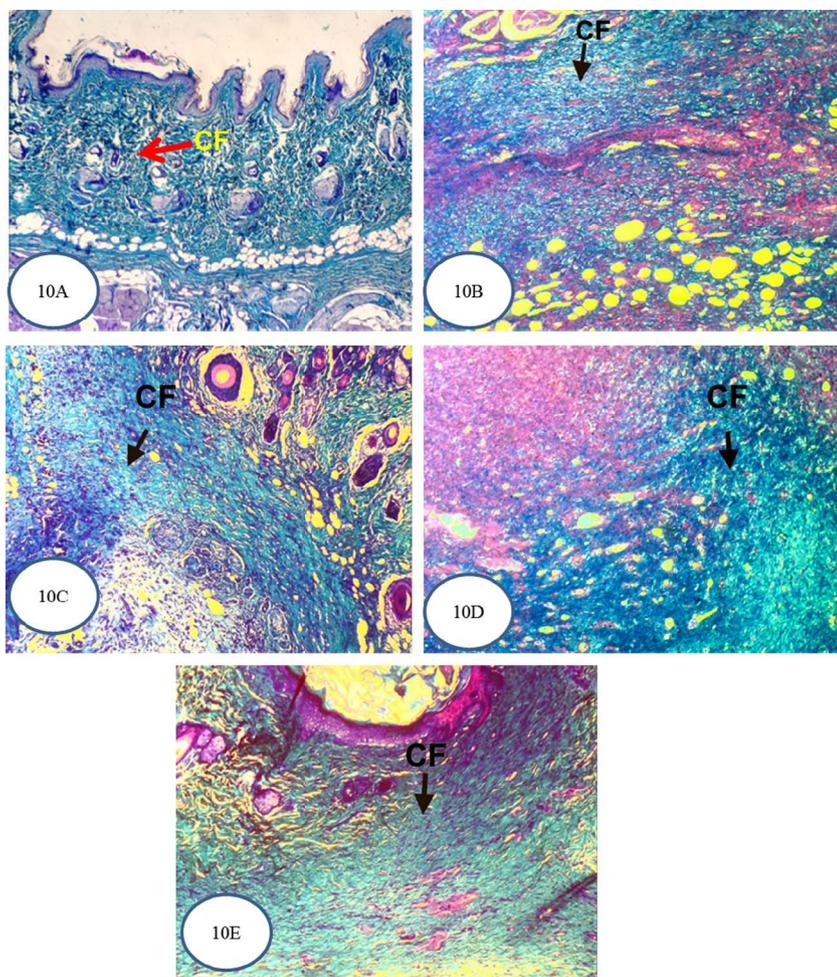


Fig. 10. Photomicrographs of Masson's Trichrome stained sections of skin of diabetic non-wounded rat (10A) and diabetic wounded rats (10B–10E) at day 14 after wounding showing collagen distribution and content in the dermis. 10A: Photomicrograph of a v. s. in skin of non-diabetic non-wounded control rat showing normal collagen fibers in the dermis. 10B: Photomicrograph of a v. s. in skin of non-diabetic wounded rat showing not well-oriented collagen fibers in the dermis. 10C: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin showing randomly oriented collagen fibers in the dermis. 10D: Photomicrograph of v. s. in skin of non-diabetic wounded rat treated with LLLT showing haphazardly arranged collagen fibers in the dermis. 10E: Photomicrograph of a v. s. in skin of non-diabetic wounded rat treated with quercetin and LLLT showing well oriented collagen fibers in the dermis. (Masson's Trichrome stain X 100).

Table 2

Effect of quercetin treatment and low level laser therapy (LLLT) on blood glucose level (mg/dl) and serum insulin level (ng/ml) in non-diabetic wounded and diabetic wounded rats.

Groups	Parameters			
	Blood glucose level (mg/dl)		Serum insulin level (ng/ml)	
	7 days	14 days	7 days	14 days
Non-diabetic non-wounded group	112.3 ± 2.9 ^{de}	92.0 ± 3.5 ^e	3.0 ± 0.4 ^{de}	2.8 ± 0.1 ^{ef}
Non-diabetic wounded control group	105 ± 4.8 ^e	108.3 ± 1.8 ^e	2.5 ± 0.1 ^{ef}	2.9 ± 0.3 ^{ef}
Non-diabetic wounded group treated with quercetin	98.1 ± 2.9 ^e	105.3 ± 6.3 ^e	4.1 ± 0.1 ^{bc}	4.7 ± 0.1 ^a
Non-diabetic wounded group treated with LLLT	104 ± 14.1 ^e	105.8 ± 3.02 ^e	3.6 ± 0.2 ^{cd}	4.5 ± 0.2 ^{ab}
Non-diabetic wounded group treated with quercetin + LLLT	111.8 ± 3.2 ^{de}	102.5 ± 3.4 ^e	3.7 ± 0.1 ^c	3.9 ± 0.2 ^c
Diabetic non-wounded group	373.3 ± 34.2 ^b	378.1 ± 46.2 ^b	1.0 ± 0.1 ^h	0.8 ± 0.3 ^{hij}
Diabetic wounded control group	474.6 ± 36.4 ^a	402.0 ± 38.1 ^b	0.3 ± 0.04 ^j	0.6 ± 0.04 ^{hij}
Diabetic wounded group treated with quercetin	136 ± 1.52 ^{de}	132.1 ± 3.6 ^{de}	0.4 ± 0.1 ^{ij}	1.9 ± 0.04 ^g
Diabetic wounded group treated with LLLT	168.8 ± 33.6 ^d	248 ± 30.9 ^c	1.1 ± 0.1 ^h	0.9 ± 0.1 ^{hi}
Diabetic wounded group treated with quercetin + LLLT	147.3 ± 7.3 ^{de}	134.5 ± 10.6 ^{de}	2.6 ± 0.2 ^{ef}	2.4 ± 0.1 ^{fg}
F- probability	P < 0.001		P < 0.001	
LSD of the 5% level	60.31		0.60	
LSD of the 1% level	81.22		0.80	

Data are expressed as mean ± standard error. Number of animal in each group is six.

For each parameter, the means which share the same superscript symbol(s) are not significantly different.

3.4. Effects of quercetin and LLLT blood glucose and serum insulin levels

The data showing the effect quercetin and/or LLLT on fasting blood glucose and serum insulin levels in non-diabetic wounded rats and in diabetic wounded rats were depicted in Table 2.

Blood glucose level was not significantly affected in non-diabetic wounded rats treated with quercetin and LLLT for 7 and 14 days as

compared to the wounded non-diabetic control. On the other hand, blood glucose level was significantly elevated ($p < 0.01$; LSD) in uninjured diabetic rats and in diabetic wounded rats as compared to the non-diabetic control rats. The treatment of diabetic wounded rats with quercetin and LLLT for 7 days or 14 days potentially ameliorated ($p < 0.01$; LSD) the elevated blood glucose levels.

The serum insulin level, on the other hand, was significantly

Table 3Effect of quercetin treatment and LLLT on serum IL-1 β and TNF- α levels in non-diabetic wounded and diabetic wounded rats.

Groups	Parameter			
	Serum IL-1 β level (Pg/ml)		Serum TNF- α level (Pg/ml)	
	7 days	14 days	7 days	14 days
Non- diabetic non-wounded group	31.4 \pm 0.7 ^m	31.4 \pm 1.6 ^m	29.9 \pm 1.8 ^{mn}	28.4 \pm 1.5 ⁿ
Non-diabetic wounded control group	66.1 \pm 2.2 ^{hij}	92.9 \pm 4.5 ^c	63.4 \pm 2.3 ⁱ	86.1 \pm 1.4 ^{fg}
Non-diabetic wounded group treated with quercetin	41.5 \pm 1.6 ^{kl}	61.7 \pm 2.8 ^{ij}	40.1 \pm 1.0 ^{lm}	61.4 \pm 3.19 ^{ij}
Non-diabetic wounded group treated with LLLT	41.5 \pm 1.6 ^{kl}	69.2 \pm 3.6 ^{hi}	39.5 \pm 2.5 ^{lm}	69.6 \pm 1.0 ^{hi}
Non-diabetic wounded group treated with quercetin + LLLT	36.6 \pm 1.5 ^{lm}	47.9 \pm 1.7 ^k	39.9 \pm 1.3 ^{lm}	49.4 \pm 2.4 ^{kl}
Diabetic non-wounded group	109.4 \pm 1.5 ^d	142.2 \pm 3.2 ^b	109.2 \pm 4.2 ^d	162.6 \pm 9.0 ^b
Diabetic wounded control group	127.6 \pm 1.0 ^c	185.7 \pm 5.9 ^a	121.9 \pm 3.2 ^c	210.8 \pm 3.4 ^a
Diabetic wounded group treated with quercetin	75.4 \pm 5.1 ^{gh}	105.5 \pm 3.1 ^d	63.8 \pm 6.5 ⁱ	98.0 \pm 2.3 ^e
Diabetic wounded group treated with LLLT	82.2 \pm 1.4 ^{fg}	104.4 \pm 4.4 ^d	39.5 \pm 2.5 ^{ij}	95.6 \pm 6.1 ^{ef}
Diabetic wounded group treated with quercetin + LLLT	58.9 \pm 6.6 ^j	86.0 \pm 6.0 ^{ef}	51.2 \pm 3.4 ^{jk}	77.1 \pm 2.7 ^{gh}
F-probability	P < 0.001		P < 0.001	
LSD of the 5% level	10.06		8.28	
LSD of the 1% level	13.55		6.15	

Data are expressed as mean \pm standard error. Number of animal in each group is six.

For each parameter, the means which share the same superscript symbol(s) are not significantly different.

Table 4

Effect of quercetin treatment and LLLT on serum PGE-2 and LTB-4 levels in non-diabetic wounded and diabetic wounded rats.

Groups	Parameter			
	serum PGE-2 level (Pg/ml)		Serum LTB-4 level (Pg/ml)	
	7 days	14 days	7 days	14 days
Non- diabetic non-wounded group	119.2 \pm 1.6 ^{jk}	115.2 \pm 1.3 ^k	184.2 \pm 41.6 ^{ghi}	209.4 \pm 34.9 ^{ghij}
Non-diabetic wounded control group	165.5 \pm 7.3 ^d	198.9 \pm 6.0 ^c	212.2 \pm 34.5 ^{fghi}	157.8 \pm 23.5 ^{hij}
Non-diabetic wounded group treated with quercetin	132.0 \pm 2.6 ^{hij}	152.7 \pm 6.3 ^{defg}	209.1 \pm 32.9 ^{fghij}	115.9 \pm 4.8 ^j
Non-diabetic wounded group treated with LLLT	128.1 \pm 1.8 ^{ijk}	166.7 \pm 5.8 ^d	358.3 \pm 16.4 ^{de}	132.4 \pm 11.6 ^{ij}
Non-diabetic wounded group treated with quercetin + LLLT	125.4 \pm 1.3 ^{ijk}	144.5 \pm 2.2 ^{fgh}	243.9 \pm 25.3 ^{fgh}	177.2 \pm 40.6 ^{ghij}
Diabetic non- wounded group	192.4 \pm 4.1 ^c	239.0 \pm 3.5 ^b	348.1 \pm 16.9 ^{de}	229.5 \pm 21.2 ^{fgh}
Diabetic wounded control group	230.1 \pm 7.8 ^b	287.1 \pm 7.5 ^a	734.0 \pm 45.7 ^b	299.6 \pm 62.7 ^{def}
Diabetic wounded group treated with quercetin	164.4 \pm 8.8 ^{de}	149.0 \pm 4.6 ^{efg}	108.3 \pm 42.5 ^a	186.7 \pm 41.9 ^{ghij}
Diabetic wounded group treated with LLLT	156.1 \pm 8.5 ^{def}	147.5 \pm 7.7 ^{fgh}	394.3 \pm 10.1 ^{cd}	479.5 \pm 27.8 ^c
Diabetic wounded group treated with quercetin + LLLT	164.7 \pm 8.4 ^{de}	136.6 \pm 3.2 ^{ghi}	269.9 \pm 24.2 ^{efg}	181.4 \pm 40.8 ^{ghij}
F- probability	P < .001		P < .001	
LSD of the 5% level	16.34		95.26	
LSD of the 1% level	22.01		128.29	

Data are expressed as mean \pm standard error. Number of animal in each group is six.

For each parameter, the means which share the same superscript symbol(s) are not significantly different.

decreased in diabetic non-wounded and diabetic wounded rats at days 7 and 14 as compared to the corresponding untreated uninjured non-diabetic groups. The treatment of non-diabetic wounded rats with quercetin or LLLT produced a significant increase of serum insulin level at both tested periods as compared to the corresponding untreated non-diabetic wounded control. The treatment of diabetic wounded rats with quercetin produced a non-significant effect at day 7 while it induced a significant increase ($p < 0.01$; LSD) in serum insulin level at day 14 as compared to diabetic wounded control. The diabetic wounded rats subjected to LLLT exhibited a significant increase ($p < 0.05$; LSD) in serum insulin level at day 7 while they showed a non-significant change at day 14. The co-treatment of diabetic wounded rats with quercetin and LLLT produced a significant increase ($p < 0.01$; LSD) of serum insulin level at days 7 and 14 as compared to the diabetic wounded control. Thus, the treatment of diabetic wounded rats with quercetin concomitant with LLLT was more effective in increasing serum insulin level than the treatment with either of each. One-way analysis of variance (ANOVA) indicated that the effects between groups on blood glucose and serum insulin levels are significant ($p < 0.001$; F-probability) throughout the experiment (Table 2).

3.5. Effects of quercetin and LLLT on serum cytokine levels

Serum IL-1 β level was significantly elevated ($p < 0.01$; LSD) in non-diabetic wounded and diabetic wounded rats on the 7 and 14 days after wounding as compared to the corresponding uninjured controls. The treatment of non-diabetic wounded rats as well as diabetic wounded rats with quercetin and/or LLLT produced a significant decrease ($p < 0.01$; LSD) of the elevated IL-1 β level as compared to the corresponding wounded controls. The serum TNF- α level exhibited the same behavioral pattern of IL-1 β level. The treatment of non-diabetic and diabetic wounded rats with quercetin in consistence with LLLT was the most effective in ameliorating the elevated IL-1 β and TNF- α levels (Table 3).

Serum PGE-2 level exhibited a significant increase ($p < 0.01$; LSD) in non-diabetic wounded and diabetic wounded rats as compared to the corresponding uninjured controls. The treatment of these wounded rats with quercetin and/or LLLT significantly decreased ($p < 0.01$; LSD) the elevated PGE-2 level at days 7 and 14 as compared to the corresponding untreated controls. The treatment with quercetin concomitant with the LLLT was the most effective in improving the elevated PGE-2 level of non-diabetic wounded and diabetic wounded rats. One-way ANOVA revealed that the effect between groups was significant

Table 5
Effect of quercetin treatment and LLLT on serum IL-10 level in non-diabetic wounded and diabetic wounded rats.

Groups	Parameter	
	Serum IL-10 (Pg/ml)	
	7 days	14 days
Non-diabetic non-wounded group	122.8 ± 3.14 ^{ab}	126.8 ± 3.34 ^a
Non-diabetic wounded control group	85.9 ± 5.27 ^h	59.6 ± 3.9 ^l
Non-diabetic wounded group treated with quercetin	107.3 ± 3.75 ^{de}	98.8 ± 3.33 ^f
Non-diabetic wounded group treated with LLLT	110.73 ± 4.81 ^{cd}	95.9 ± 13.10 ^{fg}
Non-diabetic wounded group treated with quercetin + LLLT	121.2 ± 1.74 ^{ab}	116.8 ± 2.90 ^{bc}
Diabetic non-wounded group	49.8 ± 8.19 ^k	41.03 ± 1.75 ^l
Diabetic wounded control group	34.9 ± 4.95 ^{lm}	30.43 ± 1.98 ^m
Diabetic wounded group treated with quercetin	101.5 ± 6.09 ^{ef}	186.7 ± 41.88 ⁱ
Diabetic wounded group treated with LLLT	101.8 ± 7.79 ^{ef}	47.95 ± 27.75 ^{sh}
Diabetic wounded group treated with quercetin + LLLT	117.6 ± 3.95 ^b	181.4 ± 40.82 ^f
F- probability	P < 0.001	
LSD of the 5% level	6.15	
LSD of the 1% level	8.28	

Data are expressed as mean ± standard error. Number of animal in each group is six. For each parameter, the means which share the same superscript symbol(s) are not significantly different.

(p < 0.001; F-probability) on serum PGE-2 level throughout the experiment (Table 4).

Serum LTB-4 level was significantly elevated (p < 0.01; LSD) in diabetic wounded group at day 7 after wounding as compared to the non-diabetic non-wounded and diabetic non-wounded groups despite its non-significant change at day 14 in such groups. The treatment of diabetic wounded rats with quercetin or with quercetin concomitant with LLLT produced a significant amelioration of the elevated LTB-4 level at days 7 and 14. Serum LTB-4 level was significantly decreased as a result of LLLT of diabetic wounded rats at day 7 while it was significantly elevated at day 14 as compared to the corresponding diabetic wounded control. One-way ANOVA revealed that the effect between groups was significant (p < 0.001; F-probability) on serum LTB-4 level throughout the experiment (Table 4).

In contrast with IL-1 β , TNF- α , PGE-2 and LTB-4, serum IL-10 level was significantly decreased in non-diabetic wounded and diabetic

Table 6
Effect of quercetin treatment and low level laser therapy (LLLT) on serum LPO and GSH levels in non-diabetic wounded and diabetic wounded rats.

Groups	Parameter			
	Serum LPO level (nmole/ml)		Serum GSH level (μ mole/L)	
	7 days	14 days	7 days	14 days
Non-diabetic non-wounded group	32.3 ± 1.5 ^g	30.0 ± 0.2 ^{gh}	89.2 ± 4.6 ^a	92.9 ± 5.5 ^a
Non-diabetic wounded control group	59.7 ± 5.6 ^{bcd}	51.2 ± 2.1 ^{ef}	55.2 ± 2.0 ^{cd}	28.8 ± 1.5 ^{ij}
Non-diabetic wounded group treated with quercetin	53.9 ± 2.2 ^{de}	45.7 ± 1.3 ^f	47.1 ± 5.4 ^{def}	49.4 ± 1.7 ^c
Non-diabetic wounded group treated with LLLT	60.2 ± 2.9 ^{bcd}	46.2 ± 0.3 ^f	43.6 ± 4.0 ^{efg}	54.1 ± 0.8 ^{cd}
Non-diabetic wounded group treated with quercetin + LLLT	61.6 ± 2.7 ^{bc}	49.2 ± 0.7 ^{ef}	42.2 ± 3.1 ^{fgh}	79.4 ± 1.8 ^b
Diabetic non-wounded group	65.6 ± 1.8 ^b	54.6 ± 4.3 ^{cde}	20.0 ± 0.6 ^k	24.1 ± 1.1 ^{jk}
Diabetic wounded control group	78.1 ± 6.9 ^a	55.3 ± 1.5 ^{cde}	27.7 ± 2.6 ^{ijk}	34.0 ± 4.8 ^{hi}
Diabetic wounded group treated with quercetin	24.3 ± 1.0 ^{hi}	49.7 ± 0.2 ^{ef}	35.6 ± 1.9 ^{ghi}	53.8 ± 1.1 ^{cd}
Diabetic wounded group treated with LLLT	19.7 ± 0.5 ⁱ	50.1 ± 2.1 ^{ef}	40.3 ± 3.2 ^{fgh}	51.0 ± 0.6 ^{cde}
Diabetic wounded group treated with quercetin + LLLT	19.4 ± 2.2 ⁱ	51.3 ± 1.0 ^{ef}	48.4 ± 2.6 ^{def}	55.0 ± 0.9 ^{cd}
F- probability	P < 0.001		P < 0.001	
LSD of the 5% level	7.40		8.52	
LSD of the 1% level	9.97		11.47	

Data are expressed as mean ± standard error. Number of animal in each group is six. For each parameter, the means which share the same superscript symbol(s) are not significantly different.

wounded rats. The treatment of non-diabetic wounded and diabetic wounded rats with quercetin and/or LLLT for 7 and 14 days induced a significant increase (p < 0.01; LSD) of lowered IL-10 level as compared to the corresponding controls. The treatment of non-diabetic wounded and diabetic wounded rats with quercetin concomitant with LLLT was the most effective in increasing the IL-10 level. One-way ANOVA revealed that the effect between groups was significant (p < 0.001; F-probability) on serum IL-1 β and IL-10 levels throughout the experiment (Table 5).

3.6. Effects of quercetin and LLLT on serum LPO and GSH levels

Serum LPO level was significantly increased (p < 0.01; LSD) in uninjured diabetic, non-diabetic wounded and diabetic wounded rats as compared to the uninjured controls; it was more deteriorated in the diabetic wounded rats. The treatment of non-diabetic wounded rats with quercetin produced a non-significant decrease in the elevated serum LPO level at days 7 and 14 while LLLT or LLLT concomitant with quercetin administration produced a non-significant decrease only at day 14. On the other hand, the treatments of diabetic wounded rats with quercetin, LLLT or LLLT concomitant with quercetin produced a significant (p < 0.01; LSD) decrease in the elevated serum LPO level at day 7 while they produced a non-significant decrease only at day 14. With regard to one-way ANOVA, it was revealed that the effect between groups was significant (p < 0.001) on serum LPO level throughout the experiment (Table 6).

Serum GSH level was significantly (p < 0.01; LSD) decreased in non-diabetic wounded, diabetic non-wounded and diabetic wounded rats at days 7 and 4 as compared to non-diabetic non-wounded rats. The treatment of non-diabetic wounded rats with quercetin, LLLT or quercetin and LLLT produced a significant (p < 0.01; LSD) increase in the lowered serum GSH level at day 14 as compared to non-diabetic non-wounded rats; the treatment with quercetin and LLLT seemed to be the most potent. The treatment of diabetic wounded rats with quercetin for 14 days induced a significant increase (p < 0.05; LSD) in serum GSH level while the treatment for 7 days produced a non-significant increase. On the other hand, the treatment of diabetic wounded rats with LLLT or quercetin and LLLT significantly increased the serum GSH level at both tested periods. With regard to one-way ANOVA, it was revealed that the effect between groups was significant (p < 0.001) on serum GSH level throughout the experiment (Table 6).

4. Discussion

Wound healing begins immediately after injury and proceeds by well-organized integration and interaction of various processes, growth factors and different types of cells and tissues. Wound healing process is characterized by narrowing of wound area through centripetal movement of the wound edge toward the center of the wound, producing wound closure. The process of wound healing can be divided into five phases including the cellular phase (granulation), wound contraction (narrowing of the wound area), collagenation (collagen deposition or formation of collagenous glue), epithelialization (epithelial covering) and cicatrization (scar remodeling). These phases are overlapping, and any agent which enhances the process can promote wound healing [27,28]. DM delays wound healing by affecting these processes [29,30]. Because of the side effects of many currently used chemical drugs and the delay in of wound healing in poorly controlled or uncontrolled DM, the search for potent treatment agents and strategies with less or no side effects has attracted many investigators. Thus, in the present study, a flavonoid quercetin as natural product and LLL were tested to assess their efficacies as therapies for wound healing and recovery in non-diabetic and diabetic rats.

In the present study, the wound healing, observed by morphological examination and detected by wound healing percent, was delayed in diabetic rats as compared to the non-diabetic ones and the treatment of non-diabetic wounded and diabetic wounded rats with quercetin and/or LLLT enhanced wound healing to reach 100% and 80% respectively as a result of quercetin treatment in association with LLLT. These results are in concordance with Ebaid et al. [31] and Aboud et al. [19] who stated that STZ diabetes slows wound healing and the treatment with whey protein, quercetin and LLLT hasten wound healing in both diabetic and non-diabetic rats. The enhancement of wound healing as a result of treatment of diabetic rats with quercetin and LLLT was concomitant with a potential improvement in the glycemic state and alleviation of the lowered serum insulin levels. Thus, it can be suggested that the improvement in the glycemic state and serum insulin levels may be responsible, at least in part, to the enhancement of wound healing in diabetic rats. The anti-hyperglycemic effect of quercetin in the STZ-induced diabetic rats, in the present study, is in accordance with other previous publications which stated that this phytochemical produces hypoglycemic effect in STZ-induced diabetic rats [32,33]. Furthermore, non-diabetic rats that were orally administered quercetin and subjected to LLLT daily for 7 or 14 days exhibited an enormous increase in serum insulin levels consistent with the enhanced wound healing, although fasting blood glucose levels were not significantly affected. In accordance with the present study, Tuhin et al. [34] elucidated that the healing of skin wounds was delayed significantly in diabetic rats and this delay may be correlated with the elevated plasma glucose levels in these animals. The latter authors also stated that the diabetic conditions of the rats resulted in delaying the wound healing process by abnormal physiological response. It is also worth mentioning that the enhancement of wound healing in association with increase of serum insulin level without significant changes in blood glucose level in the non-diabetic rats treated with quercetin and LLLT may reflect the important role of insulin in the wound healing process. The direct positive effect of insulin on enhancing wound healing has been unclear in previous publications. However, many investigators [35,36,37] found that insulin treatment by topical application or by injection hastened wound healing. By contrast, Weringer et al. [38] revealed that there was no detectable difference in the duration of the healing response in either insulin-treated or un-treated diabetic mice.

The histological findings of the present study revealed that the skin of the non-diabetic wounded and diabetic wounded rats exhibited marked necrosis and massive inflammatory cells infiltration at days 7 and 14 after skin injury. These histological changes in skin of wounded rats were associated with the increase in levels of serum pro-inflammatory cytokines IL-1 β and TNF- α and the levels of inflammatory

cytokines PGE-2 and LTB-4 concomitant with the decrease in the level of anti-inflammatory cytokine IL-10, reflecting preponderance of T helper cells type 1 (Th1). These cytokine levels were more deteriorated in diabetic wounded rats than in non-diabetic wounded and non-wounded diabetic rats; thereby, the worst deleterious effects on the pro-inflammatory, inflammatory and anti-inflammatory cytokines may play crucial roles in delaying wound healing in diabetic rats than in non-diabetic ones. This evidence was supported by Ebaid et al. [31] and our previous publications [9,19]. The increase in the levels of inflammatory cytokines PGE-2 and LTB-4 in wounded rats may be secondary to increase in the pro-inflammatory cytokines IL-1 β and TNF- α levels [39]. The increase in the inflammatory cytokines as a result of neutrophil infiltration during the early phase after wounding is particularly essential for debridement and the clearing of infection by the absorption of wound exudates [40,41]. Neutrophils, which are the first cells to arrive at the wound, eliminate microorganisms and then undergo apoptosis. Afterward, neutrophils are rapidly and efficiently consumed by macrophages in a process that does not lead to further inflammation [30].

The treatment of the present non-diabetic wounded and diabetic wounded rats every other day for 7 and 14 days with quercetin and LLLT led to enhancement of granular tissue formation, fibroblasts proliferation and collagen fibers production with suppression of inflammatory cells infiltration, thereby reflecting improvements in wound healing. The collagen fibers in the dermis were well oriented especially as a result of co-therapy with quercetin and LLLT. As reported by Doersch and Newell-Rogers the improvements in wounded wound healing as a result of quercetin may be due to the increase in surface α V integrin and decreased β 1 integrin [42]. This increase in surface integrin expression may be an impact factor that contributes to fibrosis including cell migration, proliferation, and extracellular matrix production.

The treatments of non-diabetic wounded and diabetic wounded rats with quercetin, LLLT or quercetin and LLLT significantly decreased the elevated IL-1 β , TNF- α , PGE-2, LTB-4 levels and significantly increased the lowered anti-inflammatory cytokine IL-10 level; the treatment with quercetin in concomitant with LLLT was the most effective. These effects led us to suggest that the treatments with quercetin and/or LLLT hastens the switch from inflammatory to anti-inflammatory responses and dominance of Th2 on Th1 during the process of wound healing. The present results are in consistency with Li et al. [43] who reported that quercetin produced its anti-inflammatory effects probably through inhibiting the production of TNF- α from macrophage and blocking TNF- α -mediated inflammation. This effect may be directly mediated *via* prevention of activating extracellular signal-related kinase (ERK), c-Jun NH₂-terminal kinase (JNK), c-Jun, and nuclear factor- κ B (NF- κ B), which are potent inducers of inflammatory gene expression and protein secretion or indirectly *via* stimulation of peroxisome proliferator-activated receptor- γ (PPAR γ) activity, thereby antagonizing NF- κ B or activator protein-1 (AP-1) transcriptional activation of inflammatory genes. In addition, quercetin has been shown to have an anti-inflammatory effect by stabilizing mast cell membranes and inhibiting histamine release from basophiles and mast cells [44,45]. The present results are in concurrent with findings of Peranteau et al. [46] who reported that overexpression of IL-10, an anti-inflammatory cytokine, decreases the inflammatory response to injury and creates an environment conducive to regenerative wound healing. Thus, based on these previously described ideas, it can be concluded that the treatment with quercetin and/or LLLT limited prolonged inflammation and modulated the immune response during the progress of wound healing in both non-diabetic and diabetic animals.

The reason for the effective acceleration of wound healing in diabetic rats using LLLT was that the absorption of laser with specific wavelength by target tissue may result in the enhancement of fibroblast proliferation and the promotion of collagen metabolism and granulation tissue formation in the diabetic wound. The major absorbing

structures for the red visible laser wavelengths are the proteins; however, the identity of the photoreceptors responsible for the biological effects of LLLT has not been resolved yet. Several studies have suggested that either elements in the mitochondrial cytochrome system or endogenous porphyrins in the cells are the energy-absorbing chromophores in LLLT [47,48]. It is important here to mention that photoenergy of 632.8 nm wavelength at the given parameters possibly induced the fibroblasts to secrete the growth factors that probably acted in an autocrine manner to increase their rate of mitosis and or reduce cell death [22]. The response of low energy laser on cells may be dose dependent as well as wavelength dependent [49]. Therefore, correct energy density with an appropriate wavelength which can be easily and safely absorbed by the targeted tissues is strongly suggested.

An increase in free radicals and diminished antioxidant activity may worsen the situation and account for the delay in wound healing and wound closure in diabetic patients. In the present study, serum LPO level was significantly increased in non-diabetic wounded, diabetic non-wounded and diabetic wounded rats as compared to the corresponding uninjured non-diabetic rats at days 7 and 14; it was more severely affected in wounded diabetic rats. These results are in concurrence with those of Ebaid et al. [9] who found that liver lipid peroxidation, which is an indicator of oxidative stress, was markedly increased in non-diabetic wounded and diabetic wounded rats as compared to the corresponding uninjured animals. The present results are also in accordance with Rosenbaum et al. [50], who suggested that oxidative stress, regardless of its source, induces cellular dysfunction in endothelial and smooth muscle cells and reduces angiogenesis and the healing process.

The treatment of wounded diabetic rats with quercetin, LLLT or quercetin and LLLT successfully improved the elevated serum LPO levels at day 7. This improvement in LPO level was associated with suppression of the inflammatory cytokines levels and amelioration of anti-inflammatory cytokine production, granular tissue formation, fibroblasts proliferation, inflammatory cells infiltration and collagen fibers production.

GSH is the main intracellular redox buffer that functions as a direct free radical scavenger, co-substrate for glutathione peroxidase and co-factor for many enzymes [51]. The notable decline in this non-enzymatic antioxidant provokes the susceptibility to oxidative stress [52,53]. In the present study, the serum GSH level was significantly decreased in wounded non-diabetic and non-wounded diabetic and wounded diabetic rats as compared to the corresponding non-wounded non-diabetic rats at days 7 and 14. These results run parallel with those of Ebaid et al. [9] who demonstrated that hepatic GSH content was markedly decreased in wounded diabetic rats. The treatment of non-diabetic wounded rats with quercetin, LLLT or quercetin and LLLT produced a significant increase in serum GSH level while the treatment of diabetic wounded rats induced a remarkable increase at both tested periods. These results are in accordance with Liu et al. [54] who reported that the mechanism of pharmacological action was related, at least in part, to the antioxidant activity of quercetin. In the same regard, Mahmoud et al. [53] demonstrated that hesperidin and naringin exhibited antidiabetic effects in a rat model of type 2 DM by potentiating the antioxidant defense system and suppressing pro-inflammatory cytokine production.

In conclusion, the co-treatment with quercetin and LLLT was more potent in enhancing wound healing in non-diabetic and diabetic rats than the treatment with either of each since they enhanced well-oriented granulation and fibrillar collagen formation. Moreover, enhanced wound healing process in non-diabetic and diabetic rats due to treatment with quercetin and LLLT may be mediated via limiting elongated inflammation, improving glycemic state, increasing insulin level, suppressing oxidative stress and enhancing the antioxidant defense system. However, further studies are required to assess the effect of quercetin and LLLT on the phases of wound healing, including granulation, collagenation, epithelialization and cicatrization at the

cellular and molecular levels.

Conflict of interest

The author declared that there is no conflict of interest.

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