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Chitosan/Heparin Complex As Efficient Strategy to Enhance Diabetic Wound Healing

Basant Sayed [1*], Abdel-Rahman B. Abdel-Ghaffer [1], Dina M. Seoudi [1], Abdel Nasser Badawi Singab [2]

[1] *Biochemistry Department, Faculty of Science, Ain shams University Cairo, Egypt.*

[2] *Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University Cairo, Egypt.*

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Correspondence
Basant Sayed
E-mail*
(Corresponding Author)
basantsayed_p@sci.asu.edu.eg

ABSTRACT

Diabetic wound healing is prevented by hyperglycemia and its complications, including impaired tissue repair and inflammatory response. Advanced glycation end products (AGEs) are critical in these complications, increasing oxidative stress and tissue damage. Chitosan, a natural polysaccharide, has shown potential in reducing AGEs and promoting tissue repair. This study evaluates the effectiveness of high viscous ointment of water-soluble chitosan (WSC)/Heparin complex in enhancing wound healing in rat models. Diabetic wounds were induced in a streptozotocin (STZ)-treated rat model. Rats were assigned to control, heparin, WSC, and WSC/heparin complex treatment groups. Blood glucose, liver enzymes, kidney markers, collagen, elastin, TGF-β, and PDGF levels were measured. Wound healing was assessed on days 5, 10, and 14, with histological evaluation for tissue morphology. Our results revealed that natural and diabetic wounds were associated with significant ($P<0.05$) elevated RBG levels, liver enzyme activities, creatinine and urea levels, and collagen, elastin, and TGF-β levels.

In contrast, they were related to reduced albumin and PDGF levels. Distinctly, DM induction was linked to significant ($P<0.05$) elevated RBG, creatinine, urea collagen, elastin, and TGF-β levels and reduced PDGF levels. This suggested the great impact of DM on inflammatory and wound healing-associated parameters. In conclusion, these results showed that the WSC/heparin complex effectively attenuated liver and kidney damage, enhanced growth factors production, decreased inflammatory infiltrate amount, induced great wound recovery, and improved morphology of diabetic wound tissues. Thus, this formula might represent a promising strategy to accelerate the healing of diabetic wounds.

1. Introduction

Wound healing is an biological important process involving an intricate sequence of biochemical and cellular responses [1, 2]. The wound healing systematic process may be explained in light of 4 overlapping phases: hemostatic, inflammatory, proliferative, and maturation or remodeling phase [3]. The hemostatic phase includes the body's immediate response to prevent blood loss via blood clot formation and vasoconstriction. The inflammatory phase is mainly related to infection prevention through the infiltration of lymphocytes, macrophages, and neutrophils. The proliferative phase initiates to repair of the defective wound through the simultaneous formation of wound contraction, collagen deposition, angiogenesis, and granulation tissue. In the maturation stage, tissue structure is regained via scar tissue maturation, normal epithelium development, and collagen rearrangement [1, 4].

Despite that, diabetes mellitus (DM) is related to many pathological changes that are associated with poor wound healing. These pathological changes may contribute to impaired collagen deposition, increased oxidative stress, reduced neovascularization, structural changes in fibroblast membranes, and a chronic inflammatory phase [5, 6]. Thus within clinical practice and medical research, diabetic wound healing stands as a multifaceted challenge and the search for interesting solutions to prevent DM-related complexities pushes researchers to recognize advanced biomaterials [7].

The delayed wound healing In the Diabetes Mellitus case is mainly associated with the hyperglycemia-induced production of advanced glycation end products (AGEs) and their related secondary complications [18]. Thus, any compound that distinctly suppresses AGE production or counteracts AGE-related complications may prove beneficial in enhancing the diabetic wound healing process [1]. Hydrocolloid macromolecules are polysaccharides that contain hydrophilic groups, such as amine, hydroxyl, and carboxy groups [19]. Recently, several reports have found that hydrocolloids can suppress potentially harmful byproducts formation, including AGEs. Among eight common hydrocolloids, chitosan showed the highest inhibitory effects on AGE formation [20].

The water-soluble derivative of chitosan (WSC) possesses many favorable wound healing features such as angiogenic and bactericidal activity, metal chelating ability, and anti-inflammatory, antioxidant, and hemostatic properties [1, 8 & 9]. WSC has the potential to regulate endothelial cells and fibroblast behavior because of its anionic groups [10, 11]. Moreover in the vicinity of the cells, WSC may immobilize several growth factors such as epidermal growth factors (EGF), fibroblast growth factor-2 (FGF2), and vascular endothelial growth factor (VEGF) [1, 12]. So, these growth factors' specific interaction with the cell surface will then lead to secretion, growth, and subsequent connective tissue regeneration [13].

On another hand, for treating chronic wounds, glycosaminoglycans represent a good option [14]. Heparin is one of the most common glycosaminoglycans that is used as an anticoagulant and has been suggested as a wound healing enhancer because of its ability to prevent main growth factors proteolysis via interaction with them [15]. Considering these WSC and heparin properties, in this study we hypothesize that the WSC+Heparin combination may be effective for DM wound healing. We evaluated their impact on improving liver and kidney functions, enhancing anti-inflammatory effects and growth factor levels (Transforming Growth Factor (TGF)-β and platelet-derived growth factor (PDGF)), and improving collagen and elastin levels in rats' models with diabetic wounds.

2. Materials and methods

2.1. Materials

Streptozotocin (STZ) (Number: S0130) was purchased from Sigma Aldrich, USA. Heparin commercial solution (5000UI) was purchased from Nile Company, Egypt. Sodium hydroxide pellet (NaOH) (RFE, USP-NF, BP, ph. Eur.), Acetic acid 100% (CH3COOH, BDH, AnalaR), Absolute ethanol (C_2H_6O) (Ph. Eur., BP, USP), Acetone (C3H6O) (BP, NF, Ph. Eur.), and Hydrochloric acid 37 % (HCL) (P.a, Reag . ACS + ISO +Ph. Eur.) were commercially obtained from Honeywell, USA.

2.1.1. Water soluble chitosan preparation

Crayfish, a freshwater crustacean belonging to the superfamilies Astacoidea, was obtained from the Nile River in Gizerat elwraq, Giza, Egypt. Carefully, samples were enclosed in plastic containers and transported to the Faculty of Science Laboratories, Cairo University. Fish shells were washed to remove residual adhesive tissue, dried for six hours in an oven (60°C), and then scraped. Using a described method by Fernandez-Kim, (2004) [16] and Omar B. A.et al. (2021) [17], a multi-step process to remove minerals, proteins, and pigments was used for the prepared Crayfish shells First, demineralization is achieved by treating the shell powder with 10% HCl at room temperature for 12 hours, followed by washing and drying. Then deproteinization is performed using 4-5% NaOH to eliminate proteins, with stirring for 12 hours before filtration, washing, and drying. The decolorization step involves soaking the shell powder in acetone at 70°C for 12 hours to remove pigments. Finally, deacetylation is carried out by reacting the chitin with 50% NaOH at 115°C for 24 hours to yield chitosan, which is then filtered, washed, and dried to a stable weight. Data regarding chitosan molecular bonds was confirmed by Fourier-transform infrared (FT-IR) spectra in the frequency range of 4000-500 cm−1 using an FT-IR spectrometer (FTIR- 8300, Shimadzu, Japan).

2.2.2. Preparation of WSC/Heparin complex

WSC powder (5g) was added into a distilled water flask (100) with stirring for one hour to dissolve well. Into the flask, Heparin solution (10 mL, 5000UI) was added slowly with stirring for one hour (200 rpm, at 40°C). After that, the solution was filtered to remove water-insoluble products. By lyophilization for 24 hours at 40°C, WSC/Heparin white powder complex was obtained. For ointment preparation WSC (2.5 g) was dissolved with WSC/Heparin complex in distilled water (50 mL) with stirring for one hour at room temperature. Then, a high viscous ointment of WSC/Heparin complex was obtained. [48]

2.2. Methods

2.2.1. Animals and experimental design

Healthy Wistar albino adult male rats (160-200g) obtained from the Faculty of Veterinary Medicine, Cairo University, Egypt were included in the experiment. All animals were maintained under standard animal house conditions and a 12-hour light/dark natural cycle.

For DM induction, rats were administered STZ intraperitoneal injection (45 mg/kg body weight) (dissolved in 0.1 M citrate buffer, pH 4) daily for five days. After that, a glucometer (Accu-check, Roche Diabetes Care, Inc. India) was used to monitor the venous blood glucose levels and when the sustained blood glucose levels exceeded 200 mg/dL, the rats were considered diabetic rats and used for the study. [49, 50]

Rats were assigned into 9 groups (4 rats/each): Group 1: normal rats received distilled water and acted as normal control; Group 2: normal rats with wounds and were not subjected to any treatments (natural healing); Group 3: DM rats with wound and were not subjected to any treatments (natural healing); Group 4: normal rats with wound were treated with WSC epicutaneous 0.5g was spread evenly throughout the area of the skin wound for 14 days; Group 5: DM rats with wound treated with WSC epicutaneous (0.5g for 14 days); Group 6: normal rats with wound treated with Heparin solution epicutaneous, drops were dropped twice evenly throughout the area of the wound for 14 days; Group 7: DM rats with wound treated with Heparin solution (drops twice for 14 days); Group 8: normal rats with wound treated with WSC\Heparin complex ointment epicutaneous (0.5g for 14 days) and finally Group 9: DM rats with wound treated with WSC\Heparin complex ointment epicutaneous (0.5g for 14 days).

2.2.2. Creation of wound and evaluation of wound contraction

In all animals, wounds were created according to the Anushree et al. method. The skin's dorsal surface was shaved and sterilized by ethanol. Then, animals were anesthetized(1.2 g/kg intraperitoneal) , and a full-thickness wound (about 2 cm2 area) was created by cutting out a piece of skin extending up to the muscle layer. Using a digital camera (Canon, 18MP), the wound contraction was assessed from the wound area photographs taken on the 0, 5th, 10th, and 14th day post-wound creation. Wound contraction (%) = wound area on day 0 – wound area on day (n) / [wound area on day 0 × 100] [1].

2.2.3. Samples collection and biochemical measurements

At the experiment's end, all rodents were sacrificed with urethane anesthesia, and, using a cardiac needle, blood samples (about 5mL) were collected.

In a kinetic manner, liver enzymes aspartate (AST) and alanine aminotransferase (ALT) activities and creatinine were measured using commercial kits (Bio-Diagnostic Company, Cairo, Egypt) according to the manufacturer's protocols. Also, random blood glucose (RBG), albumin, and urea levels were determined in a colorimetric manner using commercial kits (Bio-Diagnostic Company, Cairo, Egypt). Also according to the manufacturer's guidelines, serum levels of Elastin (ER0508) were obtained by (Fine Biotech Co., ltd., China), Collagen (NBP2-75823) was obtained by (Novus Biologicals, USA), TGF-β (SEA124Ra) was obtained by (Cloud-Clone crop, USA), PDGF was obtained by (E-EL-H1577) (Elabscience, USA).

2.2.4. Histopathological evaluation

Skin samples were obtained from rats immersed in formalin (10%, Merck Germany) for 24 hours and then dehydrated in ascending grades of ethanol concentration, cleaned in xylol, and embedded in paraffin (Merck Germany). Subsequently by using a microtome, serial sections (5µm thickness) were cut and then mounted on glass slides. All paraffin tissue sections were stained with Hematoxylin and Eosin (H&E) to evaluate the histological structures.

2.2.5. Statistical analysis

Data were analyzed by SPSS 20 (SPSS Inc, USA), and GraphPad Prism 8 (GraphPad, San Diego, CA, USA) was used to display different charts. Results were expressed as means ± standard deviation (SD). Multiple comparisons between groups were assessed using the ANOVA test followed by the Tukey test as a post-hoc test. P≤0.05 was significant.

3. Results

2.1. FTIR studies of chitosan

Results in fig. 1 showed the chemical structure of the chitosan has characteristic peaks at 711, 877,1078,1149,1391,1659,2506,2851,3309 cm^{-1}.

2.2. Impact of DM on liver, kidney, inflammatory and wound-healing parameters

Both rats with normal or diabetic wounds were associated with significant (P<0.05) elevated RBG levels, liver enzyme activities, creatinine and urea levels, and collagen, elastin, and TGF-β levels. In contrast, they were associated with decreased albumin and PDGF levels (Table 1). Compared to rats with normal wounds, DM induction was related to significant (P<0.05) elevated RBG creatinine, urea collagen, elastin, and TGF-β levels and reduced PDGF levels (Table 1). This suggested the great impact of DM on inflammatory and wound healing-associated parameters.

2.3. Attenuating effects of heparin, WSC and their complex

Although heparin and WSC administration improve RBG and liver, kidney, and anti-inflammatory functions, the impact of the WSC\Heparin complex was more remarkable and pronounced in both natural (Tables 2) and diabetic (Tables 3) healing. WSC\Heparin complex significantly (P=0.0001) reduced RBG levels, decreased activity of ALT and AST, and decreased creatinine and urea levels. Also, the WSC\Heparin complex significantly (P=0.0001) reduced collagen, elastin, and TGF-β levels while improving PDGF levels (Tables 2 and 3).

2.4. Wound analysis

After wound treatment with heparin, WSC, and WSC\Heparin complex, the wound closure rate was measured on days 5, 10, and 14. As shown in Fig. 2, the wound closure rate in both natural (Fig. 2A) and diabetic (Fig. 2B) wound healing was improved particularly using WSC and WSC\Heparin complex. In the meanwhile, the WSC\Heparin complex induced the highest wound recovery and closure rates at >80% on day 14.

2.5. WSC\Heparin complex improved wound tissue morphology

In contrast to the control wound tissue (Fig. 3A), the morphology of diabetic wounds on the 14th day of wound healing showed the formation of thin epidermis, mononuclear cellular infiltration with degenerated hair follicles and sebaceous glands that were surrounded by inflammatory cell infiltration as shown in Fig. 3B. Both heparin (Fig. 3C) and WSC (Fig. 3D) showed moderate improvement in normal wound tissue structure of the epidermis, and dermis, mild degeneration changes, and moderate increase in sebaceous glands and hair follicles with mild inflammatory cell infiltration. However, the WSC\Heparin complex showed noticeable improvement in structure and nearly normal epidermis. The dermal increase in sebaceous glands and hair follicles almost appears nearly similar to control with minimal inflammatory cell infiltration (Fig. 3E). Similar to their effects on natural healing, both heparin (Fig. 3F) and WSC (Fig. 3G) showed improved structure of the epidermis. The dermal increase in sebaceous glands and hair follicles almost appears nearly normal mild inflammatory cell infiltration. Moreover, the effect of the WSC\Heparin complex was more remarkable and pronounced (Figure 3H).

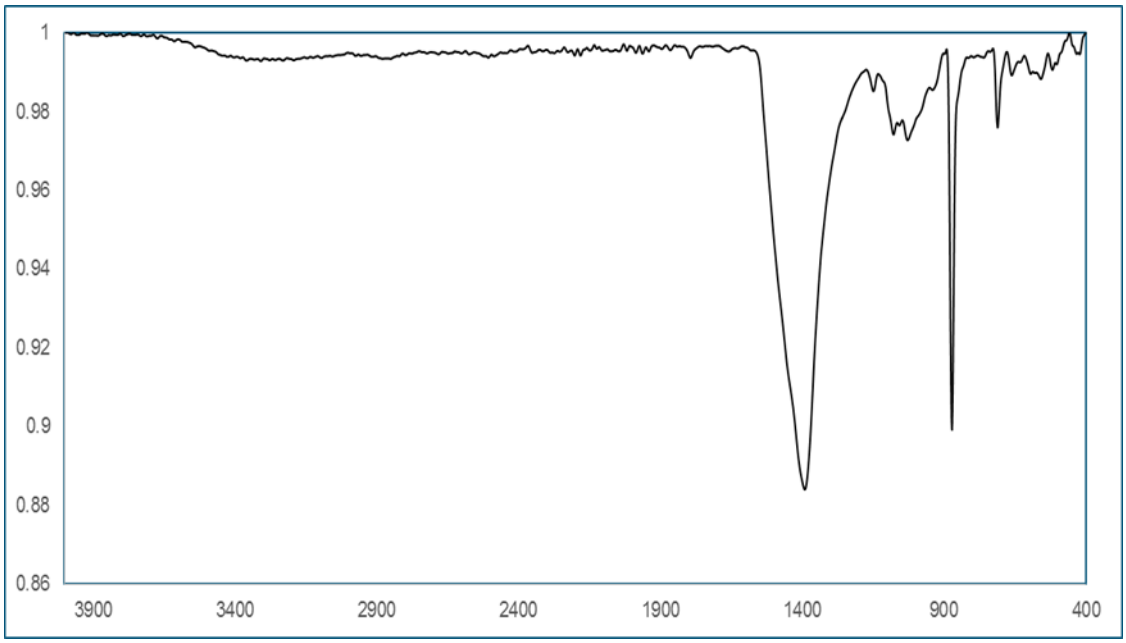

Fig. 1 FTIR of chitosan.

Table 1. Effect DM induction on liver, kidney, inflammation and wound-healing associated parameters

Variables	N	NW	DW	*P* value*	*P* value**
N. of rats	4	4	4		
RBG (mg/dL)	79.7±6.1	140.3±13.1	247.3±32.1	0.0001	0.0033
ALT (U/L)	10.7±2.1	40.7±4.3	42.8±5.3	0.0001	0.559
AST (U/L)	3.3±0.3	7.8±0.2	8.4±1.2	0.0001	0.408
Albumin (gm/dL)	49.0±5.0	22.0±4.9	18.4±4.5	0.0002	0.347
Creatinine (mg/dL)	1.7±0.2	3.2±0.2	4.3±0.3	0.0001	0.0034
Urea (mg/dL)	3.8±0.4	8.0±1.6	24.8±2.7	0.0001	0.0002
Collagen (pg/mL)	26.3±1.9	132.3±4.7	152.3±15.8	0.0001	0.0001
Elastin (ng/mL)	3.85±0.71	9.3±1.4	16.1±2.1	0.0001	0.0001
TGF-β (pg/mL)	23.9±2.2	113.9±24.2	209.8±23.3	0.0001	0.0001
PDGF (pg/mL)	485.4±25.9	215.4±17.9	176.4±21	0.0002	0.0002

N: Normal rats group , **NW:** Normal rat with Wound , **DW**: Diabetic rats with Wound Values represent mean±SD. *P*<0.05 is significant. * *P* value between three groups; ** *P* value between normal and diabetic wound groups. Differences were assessed using *ANOVA* test. RBG: Random blood glucose; ALT: alanine transaminase; AST: aspartate transaminase; TGF-β: Transforming Growth Factor-β; PDGF: Platelet-derived growth factor.

Table 2. Amelioration effects of different treatments on liver, kidney, inflammation and wound-healing associated parameters in rats with normal wound

Variables	Normal Wound	Heparin	WSC	Complex	*P* value
N. of rats	4	4	4	4	
RBG (mg/dL)	140.3±13.1	130.7±6.5	114.3±5.9	107±8.2	0.0065
ALT (U/L)	40.7±4.3	21±3	19±4.4	22.6±1.2	0.0002
AST (U/L)	7.8±0.2	5.2±0.7	3.8±0.6	4.3±0.4	0.0001
Albumin (gm/dL)	22.03±4.9	31.3±5.1	30.9±5.8	43.1±3.2	0.0051
Creatinine (mg/dL)	3.2±0.2	1.9±0.2	2.1±0.14	1.8±0.2	0.0001
Urea (mg/dL)	8.0±1.6	9.5±0.8	5.1±0.3	4.3±0.5	0.0004
Collagen (pg/mL)	132.3±4.7	122.6±9.5	107.5±8.4	48.2±8.02	0.0001
Elastin (ng/mL)	9.3±1.4	4.5±0.4	6.3±0.6	4.4±0.6	0.0004
TGF-β (pg/mL)	113.9±24.2	65.7±3.9	67.2±11	41±0.2	0.0011
PDGF (pg/mL)	215.4±17.9	330.1±40	286.1±12.9	452.4±29.3	0.0001

Values represent mean±SD. *P*<0.05 is significant. Differences were assessed using *ANOVA* test. RBG: Random blood glucose; ALT: alanine transaminase; AST: aspartate transaminase; TGF-β: Transforming Growth Factor-β; PDGF: Platelet-derived growth factor.

Table 3. Amelioration effects of different treatments on liver, kidney, inflammation and wound-healing associated parameters in rats with diabetic wound

Variables	Diabetic Wound	Heparin	WSC	Complex	*P* value
N. of rats	4	4	4	4	
RBG (mg/dL)	247.3±32.1	136.3±7.1	134.8±7.3	121±11.2	0.0001
ALT (U/L)	42.8±5.3	27.3±5.0	29.6±3.9	22.5±3.4	0.0003
AST (U/L)	8.4±1.2	5.8±0.5	5.5±0.2	4.9±0.4	0.0001
Albumin (gm/dL)	18.4±4.5	32.2±0.8	27.4±3.7	37.5±4.1	0.0001
Creatinine (mg/dL)	4.3±0.3	3.1±0.3	2.8±0.3	2.0±0.2	0.0001
Urea (mg/dL)	24.8±2.7	14.7±1.7	7.2±0.7	7.0±0.2	0.0001
Collagen (pg/mL)	152.3±15.8	88.7±14	109.6±16	68.4±10.7	0.0001
Elastin (ng/mL)	16.1±2.1	7.4±0.9	7.7±0.8	5.7±0.5	0.0001
TGF-β (pg/mL)	209.8±23.3	141.2±16.5	135.9±11.5	92±22.4	0.0001
PDGF (pg/mL)	176.4±21	269.3±25.3	358.5±49.5	436±53.5	0.0001

Values represent mean±SD. *P*<0.05 is significant. Differences were assessed using *ANOVA* test. RBG: Random blood glucose; ALT: alanine transaminase; AST: aspartate transaminase; TGF-β: Transforming Growth Factor-β; PDGF: Platelet-derived growth factor.

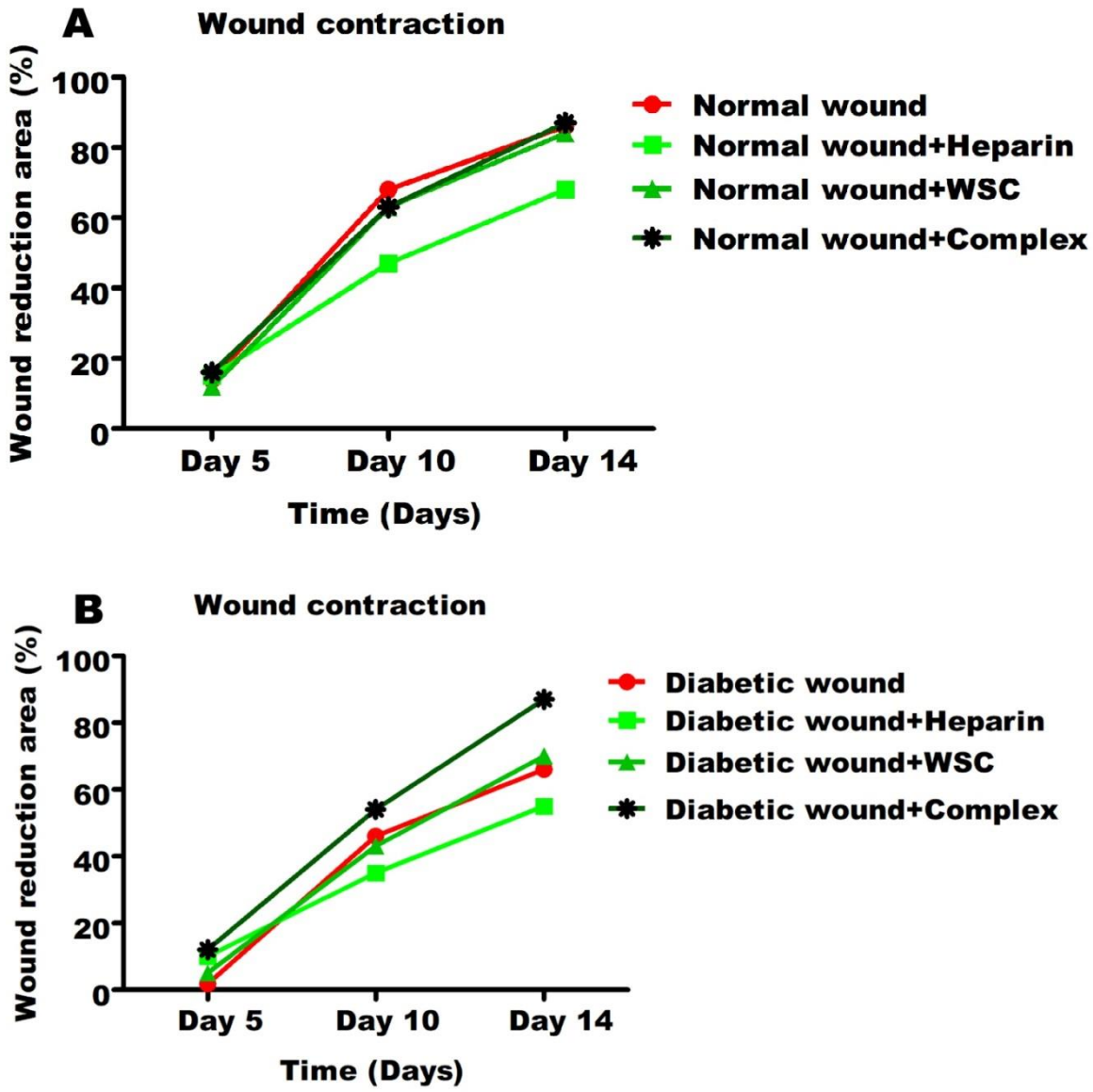

Fig. 2 The wound reduction rate was obtained on days 5, 10, and 14 after different treatments in the case of (A) natural wound and (B) diabetic wound healing. The WSC/Heparin complex induced the highest wound recovery (>80%) on day 14.

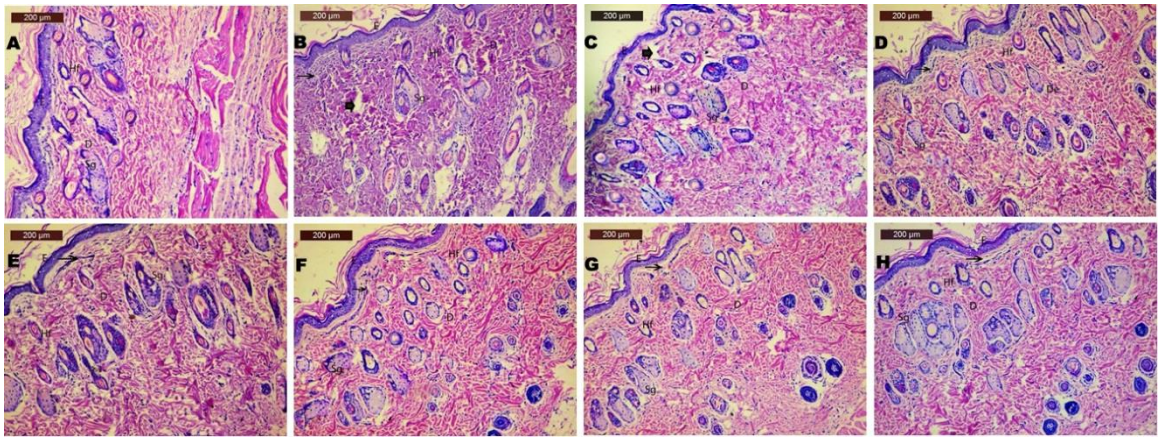

Fig. 3 Staining photomicrographs using hematoxylin-eosin (H&E) of biopsies of (**A**) the control rat skin untreated wound compared to (**B**) diabetic untreated wound. Diabetic wounds showed moderated degeneration changes, thin epidermis, and disintegrated dermis separation of epidermis basal cells with mononuclear cellular infiltration with degenerated hair follicles and sebaceous glands that were surrounded by inflammatory cell infiltration. During natural healing, both (**C**) heparin and (**D**) WSC showed moderate improvement in epidermis structure, mild degeneration changes, and a moderate increase in sebaceous glands and hair follicles with mild inflammatory cell infiltration. However, (**E**) WSC\Heparin complex showed noticeable improvement in structure and nearly normal epidermis. The dermal increase in sebaceous glands and hair follicles appears almost similar to control with minimal inflammatory cell infiltration. These effects were also seen in (**F-H**, respectively) diabetic healing and the effect of (H) WSC\Heparin complex was more remarkable and pronounced.

4. Discussion

Diabetes Mellitus causes different micro- and macro-vascular complications including diabetic kidney diseases and liver damage. In many cases, these complications are indicated by elevated activities of liver enzymes and increased creatinine and urea levels [21, 22]. It was demonstrated that chronic inflammation is closely related to type 2 DM via the release and synthesis of anti-inflammatory and proinflammatory cytokines. These cytokines, mainly TGF-β, besides adhesion molecules, are important for DM-related complications including diabetic retinopathy and nephropathy [23, 24]. Despite that, PDGF expression is decreased to some extent in DM-related chronic wounds [25]. Also owing to imbalances in the synthesis and degradation of extracellular matrix (ECM) proteins such as collagen and elastin, the skin of DM cases has greater stiffness and less flexibility, which is thought to render it more prone to injury. These alters in ECM proteins suggest the prolonged inflammatory phase, delay in granulation tissue formation, and, subsequently, decreasing wound tensile strength [26].

Our findings reported that both heparin and WSC treatment mildly improve RBG and liver, kidney, and anti-inflammatory functions. However, the WSC\Heparin complex effect was more remarkable and pronounced in both natural and diabetic healing. It reduced RBG levels, activity of ALT and AST, and creatinine and urea levels. Also, it significantly (P=0.0001) reduced collagen, elastin, and TGF-β levels while stimulating PDGF levels.

WSC has the potential to reverse diabetic hyperglycemia by increasing skeletal muscle glucose utility and uptake and decreasing hepatic gluconeogenesis [27]. Also, its anti-diabetic potential may be related to glucose homeostasis regulation [28], pancreatic β-Cells protection and insulin secretion promotion [29], and resolving insulin and leptin resistances [30]. WSC's therapeutic effect against liver damage was reported in many hepatic disease models including fibrosis [31] and non-alcoholic fatty liver disease [32]. It effectively reduced serum levels of AST and ALT [31, 33]. Its hepatoprotective role was reported in wound [34] and non-wound [33] models. Moreover, chitosan has potential attenuating effects against oxidative stress in the kidney tissue and resolves renal damage [35, 36]. In diabetic wound healing, chitosan has effective potential in reducing creatinine, uric acid, and urea levels [37, 38].

Importantly and similar to our results, chitosan was reported to reduce fibrosis proteins such as collagen, elastin, fibronectin, E-cadherin, and α-smooth muscle actin (α-SMA) for ameliorating experimental hepatic [31] and renal [39] fibrosis in vivo models. The accumulation of such ECM proteins is known to be promoted via TGF-β [40]. In agreement with our results, oral administration of insulin-loaded chitosan nanoparticles was reported to reduce serum TGF-β levels [41]. Other studies have suggested that polydatin treatment could increase renal antioxidant activity and decrease lipid peroxidation levels by reducing reactive oxygen species (ROS) production, which thus inhibits TGF-β overproduction [42].

During blood clotting, PDGF is a polypeptide released by platelets after degranulation and can stimulate cell proliferation of gingival fibroblasts and mesenchymal cells. Such growth factors interact with fibroblasts during wound healing and a great number of genes are transcribed and involved in cell proliferation [43]. In non-diabetic and diabetic wound models, several reports have shown that PDGF promotes healing by increasing wound closure and accelerating healing [44, 45]. It was reported that chitosan increases serum PDGF levels [46] and has a synergistic effect with PDGF in the wound healing process [47].

Another important finding in our study is that WSC\Heparin complex induced the highest wound recovery (>80%) on day 14 after wound treatment in both natural and diabetic wounds. As revealed by histological evaluation, the WSC\Heparin complex showed noticeable improvement in structure and nearly normal epidermis there was a dermal increase in sebaceous glands and hair follicles almost appeared nearly similar to control with minimal inflammatory cell infiltration. In the early remodeling phase and late proliferative phase, WSC was reported to have an important role that is reflected in increased fibroblast number, epidermal proliferation, angiogenesis, and collagen deposition. This role may be related to the low molecular weight WSC once placed on the wound, adheres to endothelial cells, keratinocytes, and the fibroblasts via electrostatic interactions and favors smooth proliferation and migration. Also around these cells, WSC's amphoteric nature can immobilize growth factors, which can further promote these cell's growth. Overall, these WSC features may bring about good interactions of keratinocytes, endothelial cells, and fibroblasts with their respective growth factors ultimately causing efficient epidermal regeneration, angiogenesis, and collagen deposition [1].

5. Conclusion

Our obtained results show that water-soluble chitosan has a significant impact on the wound-healing process in both natural and diabetic cases. Its complex with heparin effectively attenuated liver and kidney damage associated with DM, enhanced growth factors production, decreased inflammatory infiltrate amount, induced great wound recovery, and improved morphology of diabetic wound tissues. Thus, the WSC/heparin complex might represent a promising strategy to accelerate the healing of diabetic wounds.

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