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# The wound healing effects of the Tilapia collagen peptide mixture TY001 in streptozotocin diabetic mice

Xiaoyun Xiong[a], Jun Liang[a], Yiqiao Xu[b], Jie Liu[c], Yi Liu[d*]

[a]Yabao Pharmaceutical Group Co., Ltd, Fenglingdu, Shanxi, 044602, China;

Xiongxiaoyun@yabaoyaoye.com (X.X.); liangjun@yabaoyaoye.com (J.L.);

[b]Hunter Biotechnology, Inc, Hangzhou, China; 250943424@qq.com (Y.X.)

[c]Zunyi Medical University, Zunyi, China; Jieliu@zmc.edu.cn (J.L.)

[d]The Center for Disease Control and Prevention of Shaanxi Province, Xi'an, Shaanxi, China;

1243646864@qq.com or sxjmkly@126.com.

*Corresponding author

Yi Liu: The Center for Disease Control and Prevention of Shaanxi Province, Xi'an, Shaanxi, 710054, China, E-mail: 1243646864@qq.com or sxjmkly@126.com; Tel: 86-13700275238

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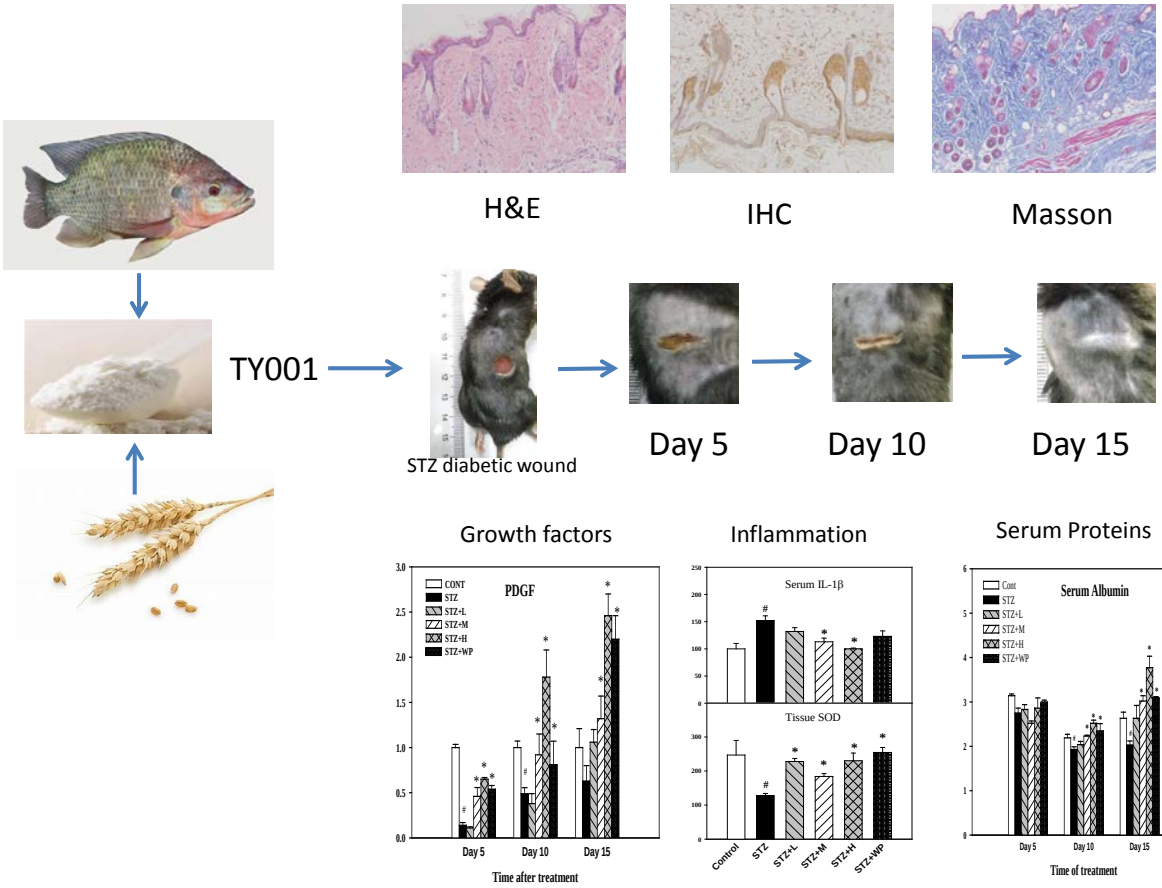
Graphic abstract




**Highlights:**

- **Collagen peptide mixture TY001 promoted STZ diabetic wound healing**
- **Collagen peptide mixture TY001 improved glucose metabolism in diabetic mice**
- **Collagen peptide mixture TY001 increased growth factor expression**

- **Collagen peptide mixture TY001 reduced inflammation in STZ diabetic mice**
- **Collagen peptide mixture TY001 improved serum protein profiles**



**Abbreviations**

TY001 Tilapia collagen peptides mixture

IGF-1 Insulin-like growth factor 1 (IGF-1)

FGF2 basic fibroblast growth factor-2

PDGF Platelet-derived growth factor

TGF-β Transforming growth factor beta

FGF Fibroblast growth factor

VEGF Vascular endothelial growth factor

EGF Epidermal growth factor

SOD Superoxide dismutase

CAT catalase

**Abstract**

The Tilapia collagen peptides mixture TY001 is effective in promoting wound healing in acetic acid-induced skin lesions in Zebrafish and in protecting against lipopolysaccharide-induced inflammation and disrupt of glucose metabolism in mice. The goal of the present study is to further examine the wound healing effects of TY001 in streptozotocin-induced diabetic mice. Full-thickness skin excision wounds were created with 8-mm biopsy punches and TY001 was administered via the drinking water (15, 30, and 45 g/L in emulsion) for 15 days. Wound healing was delayed in diabetic mice but was promoted by TY001 after 5, 10, or 15 days of treatment. Collagen deposition and tissue hydroxyproline contents were increased by TY001. The expressions of insulin growth factor-1, basic fibroblast growth factor, platelet-derived growth factor, transforming growth facts β1, vascular endothelial growth factor, and epidermal growth factor were increased by TY001 as evidenced by immunobiochemistry and qPCR. Diabetes-associated serum pro-inflammatory cytokines IL-1β and IL-8 were decreased, while anti-inflammatory IL-10 and nitric oxide were increased by TY001, along with increased tissue antioxidant superoxide dismutase and catalase activities. Diabetes-reduced serum protein levels were also recovered by TY001. Taken together, Tilapia collagen peptide mixture TY001 was effective in enhancing diabetes-associated wound healing delay, probably via increasing growth factors and collagen deposition in the wound, attenuating diabetes-induced prolonged inflammation, increasing tissue antioxidants, and providing nutritional supports in diabetic mice.

**Keywords:** Collagen peptides mixture TY001; streptozotocin diabetic mice; wound healing; growth factors; inflammatory cytokines; tissue antioxidants.

## Introduction

Wound healing is a complex physiological process involving inflammation, removal of dead cells, fibroblasts proliferation, secretion of growth factors, tissue regeneration, and remodeling [1]. In healthy person, normal wound healing occurs with three distinct phrases: Phase 1, inflammation to clean dead cells; Phase 2, migration and proliferation of different cell types for angiogenesis, granules formation, extracellular matrix deposition, and re-epithelization; and Phase 3, tissue remodeling. Under diabetic conditions, wound healing is stalled in the inflammatory phase with increased inflammatory cytokines, increased reactive oxygen species (ROS), and cellular dysfunction to form a vicious cycle of prolonged inflammation and microenvironment dysregulation [2, 3]. Under diabetic conditions, the expressions of growth factors are suppressed, further delaying wound healing [2, 4, 5]. Therefore, searching for effective approaches to improve diabetic wound healing, to reduce diabetic complications, and to provide quality of life for diabetes patients are of basic and clinical significance.

Marine collagen peptides are emerging as a potential therapeutic target for type II diabetes mellitus [6]. The collagen peptides isolated from sea cucumbers [7], Chum salmon [8], Tilapia [9, 10] have been shown to enhance wound healing in a variety of experimental models, including diabetic animals, and these collagen peptides, together with whey protein, and other natural products could act as novel immunological approaches for wound healing [11-13].

Wound healing is facilitated by proteins to perform repair process. Here we developed a unique collagen peptide mixture TY001 from Tilapia, which is the mixture of both animal and herbal peptides with high content of protein, aimed to compensate the shortage of essential amino acids [14]. TY001 has been shown to have many other beneficial effects such as the protection against LPS-induced inflammation and disruption of glucose metabolism and circadian rhythms [15], and has been in clinical trial for diabetic patients and patients with malnutrition [14], The efficacy of TY001 in promoting wound healing in acetic acid-induced Zebrafish skin lesions promoted us to hypothesize that TY001 could also be effective in diabetic wound healing.

The goal of this study is to extend our novel findings from Zebrafish to mammals, using streptozotocin (STZ)-induced mouse diabetes model, and the full-thickness skin excisions were created with biopsy punches. TY001 was provided through the drinking water based on clinical doses and prior publication. Comprehensive measures were made from wound healing to blood glucose and insulin levels, from histopathology to immunohistochemistry, from wound tissue growth factors to collagen deposition; from serum cytokine levels to tissue antioxidant enzyme activity, from serum protein profiles to nitric acid levels, the current study clearly demonstrated that supplement of TY001 in the drinking water was effective for wound healing in diabetic mice.

## 2. Materials and Methods

### 2.1. Tilapia collagen peptide TY001 and Animals

The Tilapia collagen peptide mixture TY001 (patent granted by SIPO: 2017114143555) was provided by Yabao Pharmaceutical Group Co, Ltd. (Shanxi, China). TY001 composed of Whey protein concentrate, hydrolyzed wheat protein, fish collagen, calcium caseinate, wheat oligopeptide, and casein hydrolyzed peptide as described [14]. TY001 was dissolved in the drinking water as emulsions at the concentration of 15, 30, and 45 g/L.

Three-month-old male C57BL/6 mice (25 ± 2 g) were obtained from the 4th Military University (Xi'an, Shaanxi, China). Mice were housed in the animal facility under standard conditions with 12/12 light-dark cycle, 50 ± 15% humidity, 22 ± 2°C temperature) and had free access to a standard rodent diet (AIN-93M). All of the experimental procedures were followed the Guide for the Care and Use of Laboratory Animals: Eighth Edition, ISBN-10: 0-309-15396-4, and the animal protocols were in accordance with the Animal Welfare of Chinese Guidelines and approved by the Animal Use and Care Committee at Xi'an Jiaotong University. All surgeries were performed under anesthesia and all efforts were made to minimize suffering.

**2.2. Diabetic wound model**

STZ-induced diabetic model was established by intraperitoneal injection of STZ (50 mg/kg in citrate buffer) or 0.1 mM citrate buffer, pH 4.5, 10 mL/kg), daily for consecutive 5 days, one week after the last STZ injection, tail blood was taken for blood glucose determination, and the criteria for diabetes was blood glucose > 250 mg/dL [16].

Skin biopsy puncher (8 mm in diameter) was used to produce identical whole skin-thickness wound under sterile conditions. Mice were housed individually with normal rodent chow, free access to the water. The wound healing was observed every 5 days.

**2.3. Experimental design**

Diabetic mice were divided into 5 groups: Model, TY001 low dose (L), middle dose (M), and high dose (H), and Whey protein (WP). The dose selection is based on the recommend clinical dose (20 g x 2 = 40 g for 60 kg/day), i.e, L = 20 g/kg, M =40 g/kg, and H =60 g/kg, and whey protein = 60 g/kg. Take the mouse/human convention faction of 9.1, and under the assumption that the body weight of mice was 25 g, and drunk 3-4 mL water/day, the low dose of TY001 (15 g/L) was equal to 18.92 – 25.28 g/kg, the middle dose (30 g/L) was equal to 37.92 - 50.56 g/kg, and the high dose (45 g/L) was equal to 56.88 – 75.84 g/kg. Whey protein (45 g/L) was used as positive controls, equal to the high dose of TY001. Water was freshly prepared daily in sterile drinking water bottle.

Wound healing rates were calculated by the formula: Wound healing ratio = (initial wound area – non-healing area)/initial wound area. The epithelization was considered as the complete healing. At 5, 10, 15 days after the treatment, 6 mice/group were killed to collect wound-surrounding tissues for analysis.

**2.4. Oral glucose tolerance and fasting blood glucose**

At the day 15, glucose tolerance test (GTT), and Fasting blood glucose levels (FBG) were examined before collecting tissues via commercial glucose measurement strips.

**2.5. Histopathology**

Portion of the wound-surrounding whole thickness skin (0.5 mm) was fixed in 4% paraformaldehyde, embedded in paraffin, and cut 5 μm for standard H&E, Masson trichrome stain, and observed under light microscope for inflammatory cell infiltration, angiogenesis, collagen fiber formation, fibroblasts proliferation and epithelization.

**2.6. Histoimmunochemistry**

Sections were dewaxed in Xylene, and rehydrated in graded alcohols, and in 10 mM citrate buffer, microwaved for 10 min to expose antigen, followed by 3% H2O2 to block endogenous hydroperoxides. Slides were then incubated with rabbit primary antibodies against FGF2 (Cell Signaling Technology, Shanghai, China, 1:1000), IGF-1 (CST, 1:1000) at 4 C overnight, after washing with PBS, the 2nd antibody (1:1000) was added. DAB to visualize the stain and observed under light microscope.

**2.7. Real-time RT-PCR**

Wound-surrounding tissues (50 mg) were homogenized with Trizol (TaKaRa Biotechnology Co., Ltd., Dalian, China). The quality and quantity of total RNA were determined by measuring the absorbance at wavelengths of the 260/280 nm ratio. The total RNA was reverse

transcribed to cDNA with the PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). The IQ$^{TM}$ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) was used for real-time RT-PCR analysis. PCR reaction system is 20 μL contained 10 μL iQTM SYBR Green Supermix, 1.6 μL primer mix (10 μM each), 6.4 μL ddH2O and 2 μL cDNA (10 ng/μL). The thermocycling conditions were as follows: 5 min denaturation at 95˚C; 40 cycles of annealing and extension at 60˚C for 45 sec, and denaturation at 95˚C for 15 sec. Dissociation curve was performed following the 40 cycles to verify the quality of primers and amplification. Primers were synthesized by AuGCT BioTech (Beijing, China). Relative expression of genes was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to the house keeping gene β-actin, and the relative transcript levels were calculated as percentage of the housekeeping gene.

**2.8. Serum cytokines and protein determination**

Serum IL-1β (xl-Em0187), IL-8 (xl-Em0199), and IL-10 (xl-Em0201) ELISA levels were determined via commercial kits from Shanghai XinLe Biological (Shanghai, China), according the instructions.

Serum total protein, albumin, pre-albumin, transferrin, and hydroxyproline kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), according to manufacturer's instructions.

**2.9. Tissue SOD and CAT determination**

At day 15 of the treatments, remove wound tissues and homogenized in PBS, and activities of superoxide dismutase (SOD) and catalase (CAT) were determined via commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), according to manufacturer's instructions.

### 2.10. Statistics

The software SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Data were expressed as the mean ± SEM. Age associated differences were analyzed by one-way analysis of variance, followed by the least significant difference post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

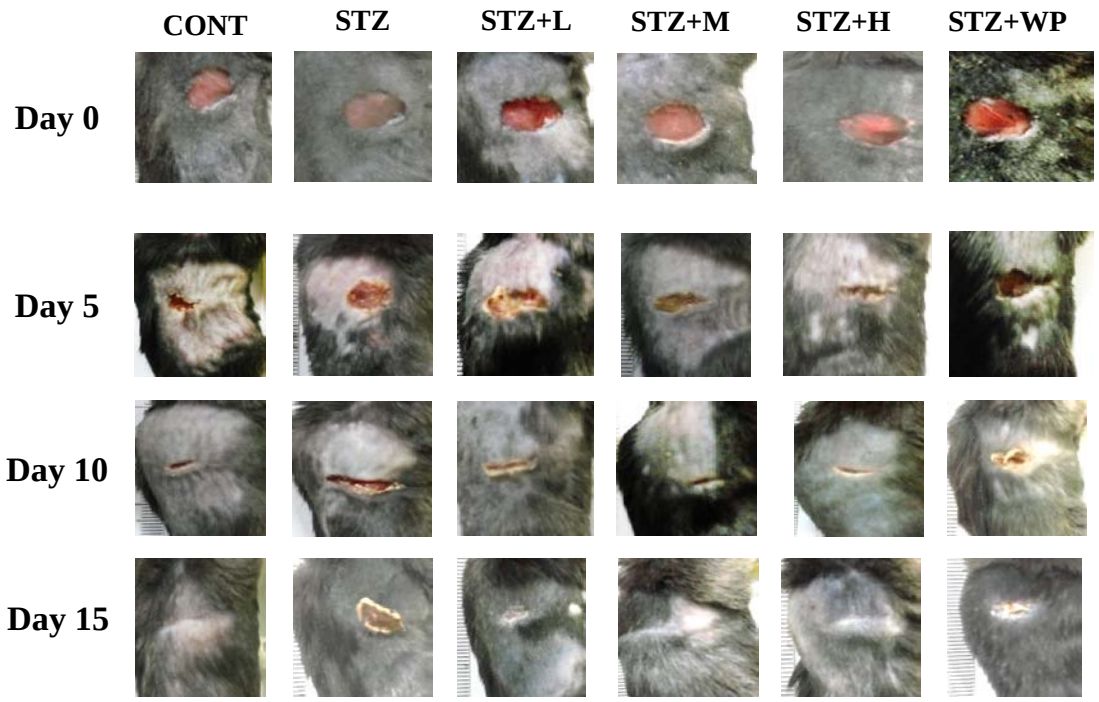
## 3. Results

### 3.1 TY001 enhanced wound healing in STZ diabetic mice

The effects of the collagen mixture TY001 on wound healing in STZ mice are shown in Figure 1. Representative images showed that the mechanical wound area is decreased with time (Figure 1A). As early as day 5 of the treatment, TY001 was effective in promoting wound healing, and the efficacy of TY001 high dose was better than whey protein (WP) with the healing rates of 20.65 ± 1.45%, 31.03 ± 3.04%，43.56 ± 3.78%，59.45 ± 5.93%, and 43.56 ± 3.78% for STZ, STZ+L (15 g/L), STZ+M (30 g/L), STZ+H (45 g/L), and STZ+WP (45 g/L),

respectively. On the day 10 of the treatment, the pink regenerative tissues appeared in STZ+H group. The wound healing rates were 32.48 ± 3.95%, 69.01 ± 5.76%, 79.08 ± 7.23%, 82.05 ± 5.92%, and 52.48 ± 3.95%. On day 15, the wound healing rates were 51.56 ± 4.92%, 87.24 ± 6.03%, 89.34 ± 8.23%, 91.34 ± 6.87%, and 51.56 ± 4.92% for STZ, STZ+L, STZ+M, STZ+H, and STZ+WP, respectively. The STZ+H group can be considered complete healing, indicating that TY001 was effective in promoting wound healing in STZ-induced diabetic mice, much better than the same dose of WP (Figure 1B).

**A**



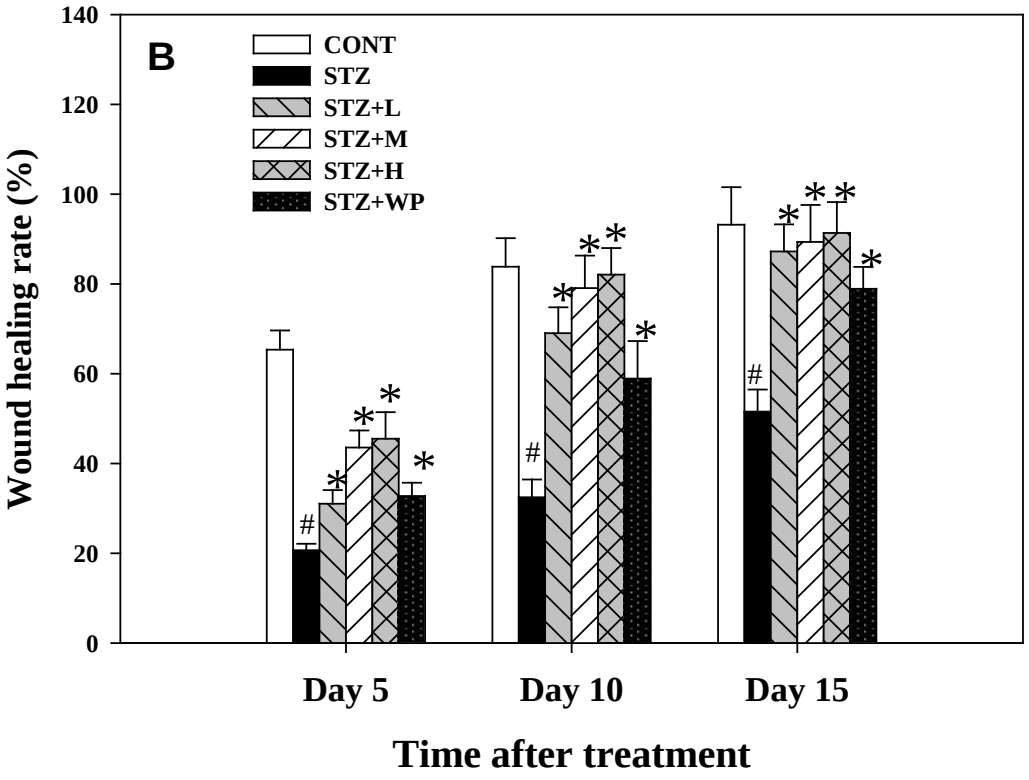
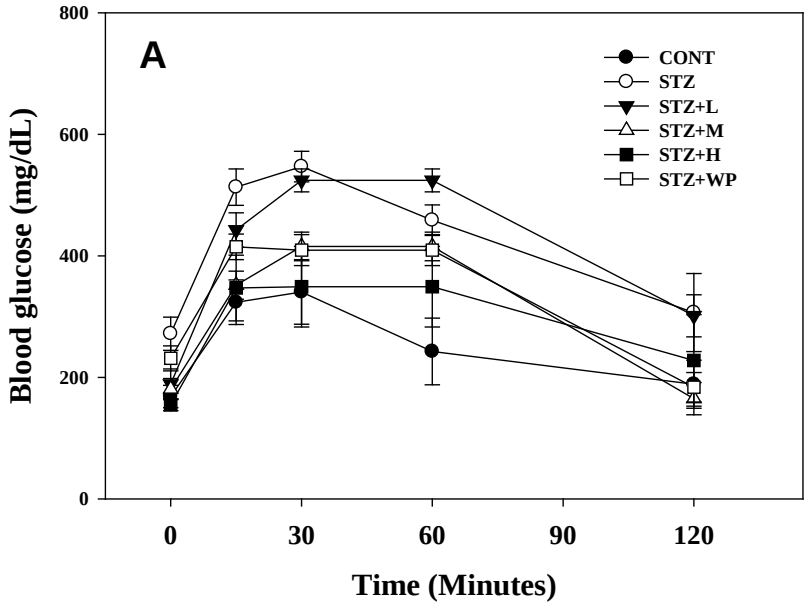


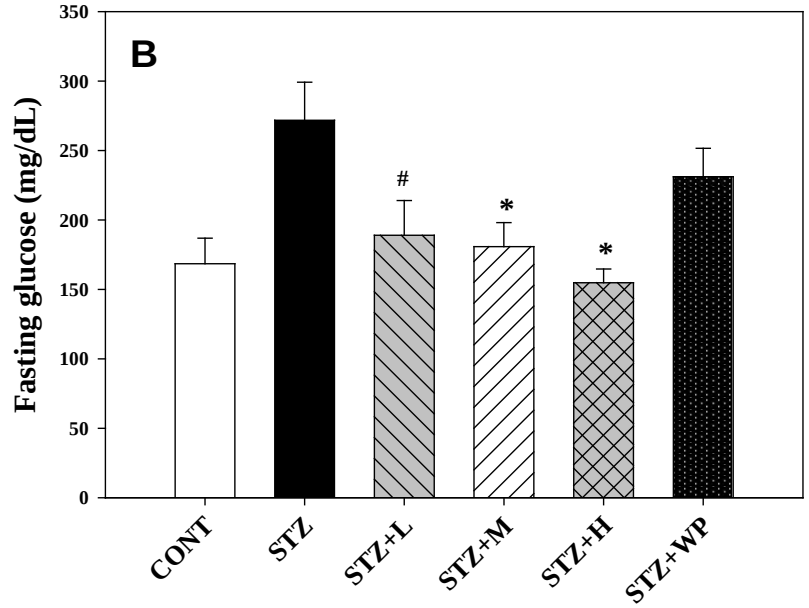

Figure 1. Effects of the collagen mixture TY001 on wound healing in STZ-induced diabetic mice. A, Representative mouse skin wound images at 5, 10, and 15 days of treatment; B, Wound healing rates (Mean ± SEM, n=6). #significantly different between Control mice and STZ mice, $p < 0.05$; *Significantly different between STZ model mice and TY001 (L, 15 g/L, M, 30 g/L, and H, 45 g/L) and whey protein (WP, 45 g/L), $p < 0.05$.

### 3.2 TY001 improved glucose metabolism in STZ diabetic mice

On day 15 of the treatment, STZ diabetic mice showed impaired glucose tolerance, and increased blood glucose took much longer time to return to the normal (Supplemental Fig. 1A)

and elevation in fasting blood glucose levels (Supplemental Fig. 1B). TY001 was effective in improving STZ-delayed glucose tolerance, and in STZ-elevated fasting blood glucose levels.

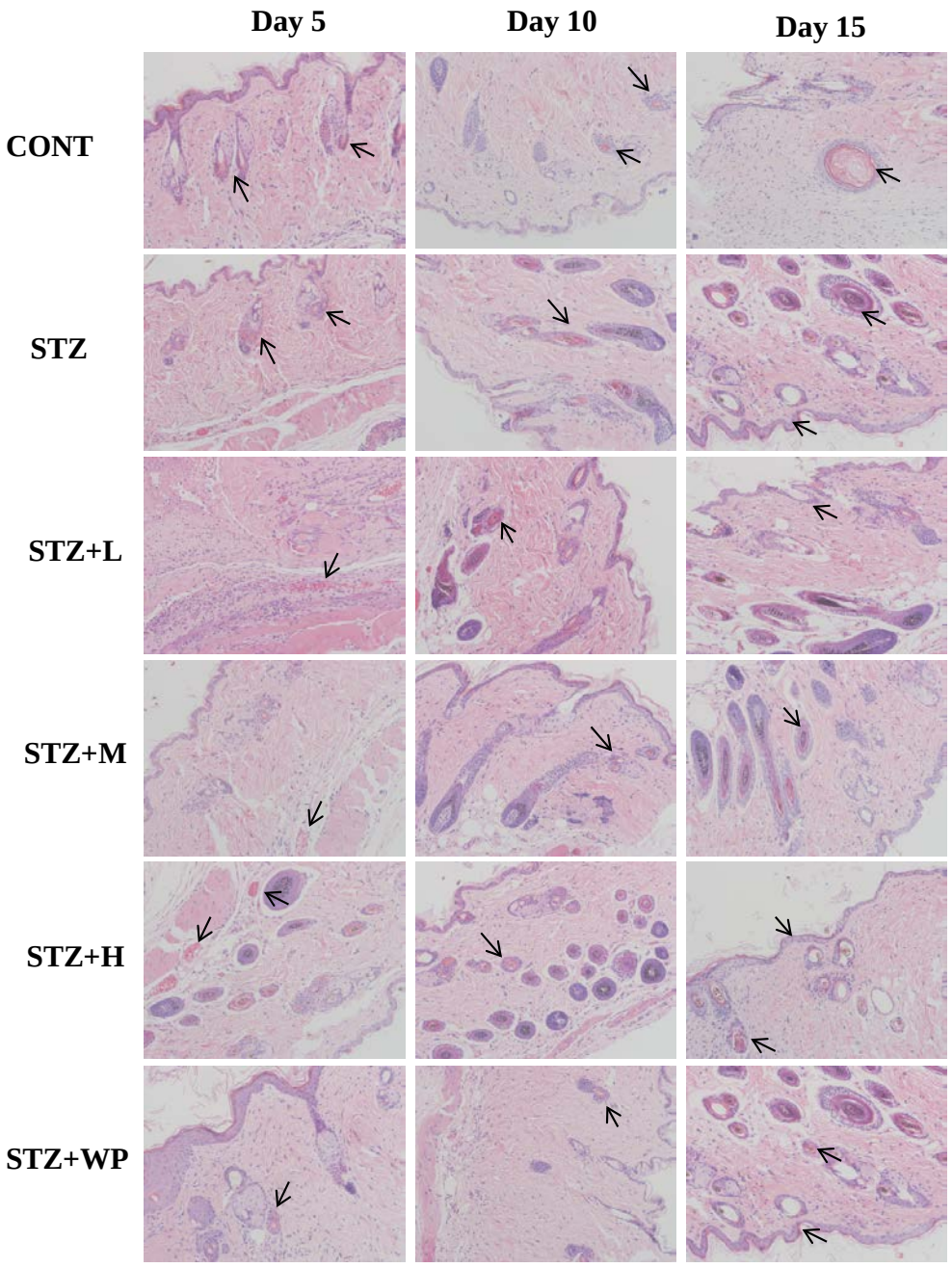


Supplemental Figure 1. On day 15 of the treatment, TY001 improved diabetes-induced glucose metabolism. A, oral glucose tolerance test; B, Fasting blood glucose levels. Data are mean ± SEM, n=6. #significantly different between Control mice and STZ mice, $p < 0.05$; *Significantly different between STZ model mice and TY001 (L, 15 g/L, M, 30 g/L, and H, 45 g/L) and whey protein (WP, 45 g/L), $p < 0.05$.

### 3.3 TY001 reduced STZ-induced wound lesions

Supplemental Figure 2 shows the standard morphology with the H&E stain. The standard H&E stain showed that in STZ group, the wound surface on day 15 of the treatment had inflammatory cell infiltration, a lot of scar formation with less capillary formation. TY001 treatment apparently improved the pathological lesions, with reduced inflammatory cells and increased fibroblasts (arrowhead), and the smooth wound surface as compared to STZ group.

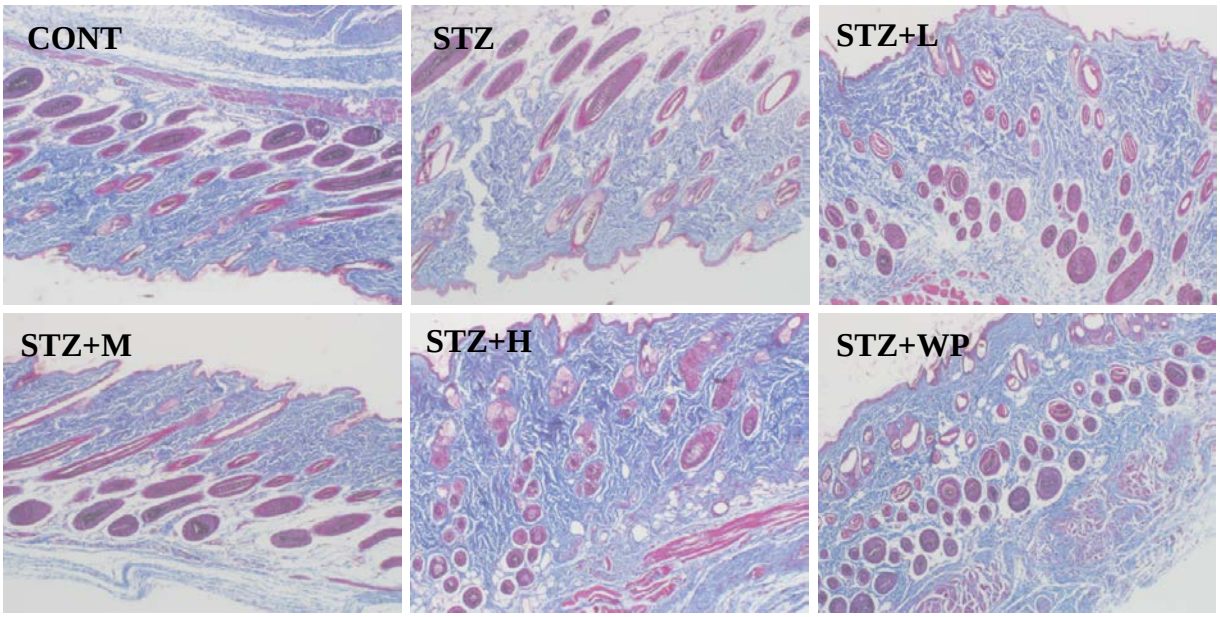


Supplemental Figure 2. Representative H&E photos at 5, 10, and 15 days after TY001 treatment. TY001 treatment apparently improved the pathological lesions, with reduced inflammatory cells and increased fibroblasts (arrowhead), and the smooth wound surface as compared to STZ group. Magnitude (100 x)

### 3.4. TY001 enhanced collagen deposition and hydroxyproline levels in the wound

The proliferation of collage fiber, i.e, collagen deposition, is the key event of granulation, which can be examined by the Masson trichrome stain. The collagen fiber is stained blue, muscle fiber red, and nuclear brown. After 15 days of treatment, collagen fibers were much more abundant in TY001 treatment groups as compared with STZ alone group, which had less collagen fibers and arranged irregularly. In TY001 groups, the collagen fibers were densely and regularly arranged, indicating that TY001 could promote collagen fiber deposition (Figure 2A). Hydroxyproline levels are regarded as an indicator of tissue collagen deposition [16]. STZ decreased tissue hydroxyproline levels, while TY001 and WP increased tissue hydroxyproline levels (Figure 2B).

A



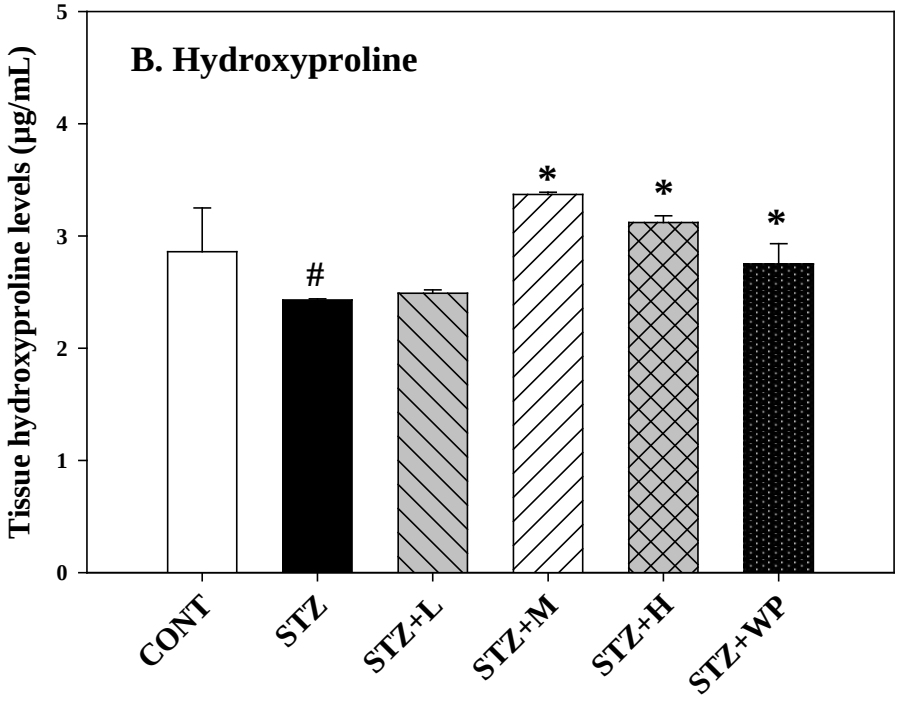



Figure 2. Effects of TY001 on skin collage contents. A. Fifteen days after treatment, the wound-surrounding skin was stained with Masson trichrome stain, magnitude (100 X). B. Tissue hydroxyproline levels. Data are mean ± SE of 6 mice. #Significantly different from controls, $p < 0.05$; *Significantly different between STZ and STZ+TY001, STZ+WP groups, $p < 0.05$.

### 3.5. TY001 enhanced insulin-like growth factor in the wound

To examine the mechanisms of promoted wound healing by TY001, the effects of TY001 on growth factors were examined. Figure 3 shows effects of TY001 on Insulin-like growth factor 1 (IGF-1) immunostaining in wound-surrounding skin tissues in STZ-induced diabetic mice on day 15 of the treatment. The yellow-brown staining indicates the positive staining of IGF-1. On day 5 of the treatment, the IGF-1 staining in wound tissue of TY001 treated mice appeared and increased on day 10 and day 15 of treatment, much higher than STZ group (Figure 3).

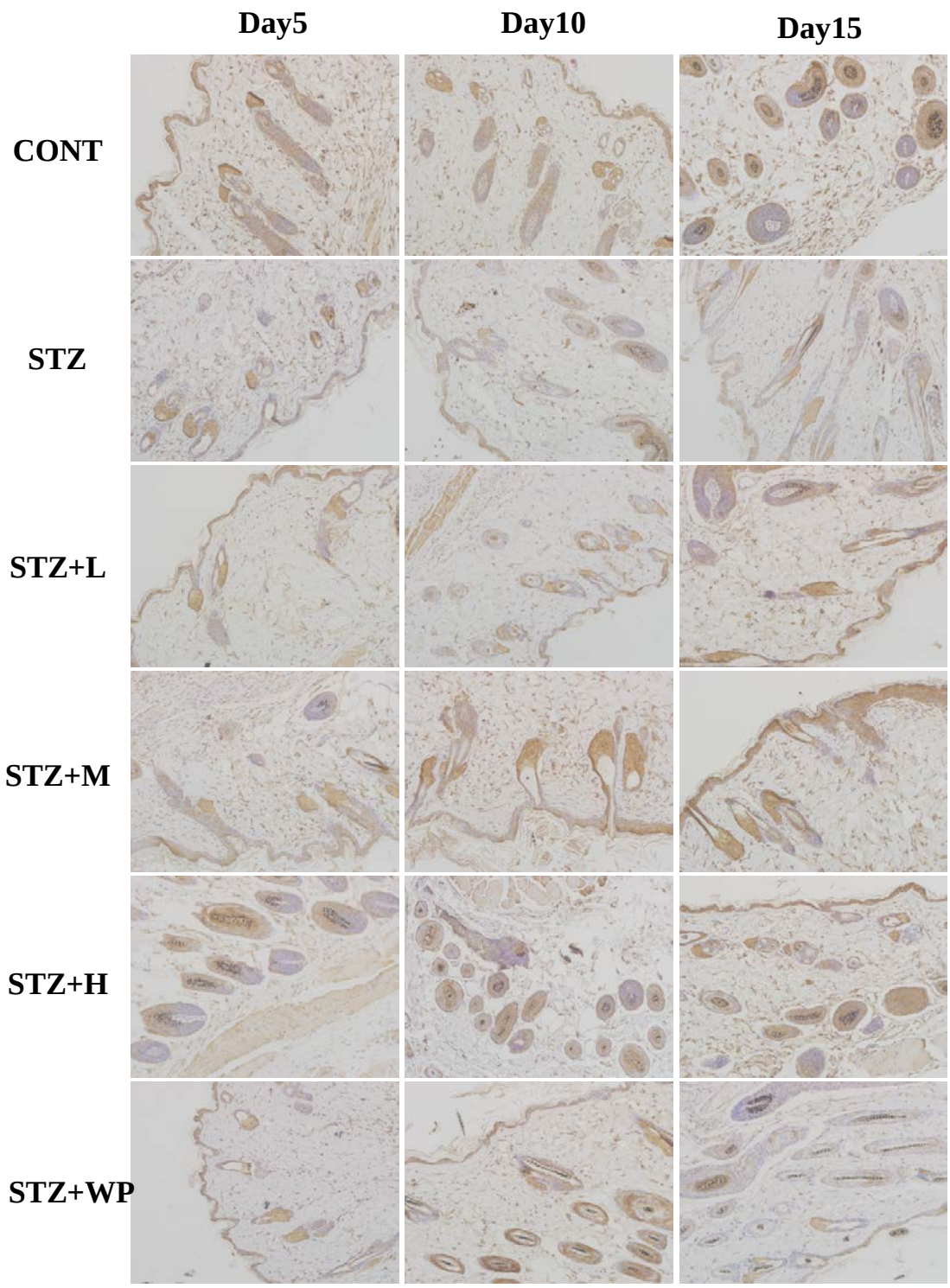
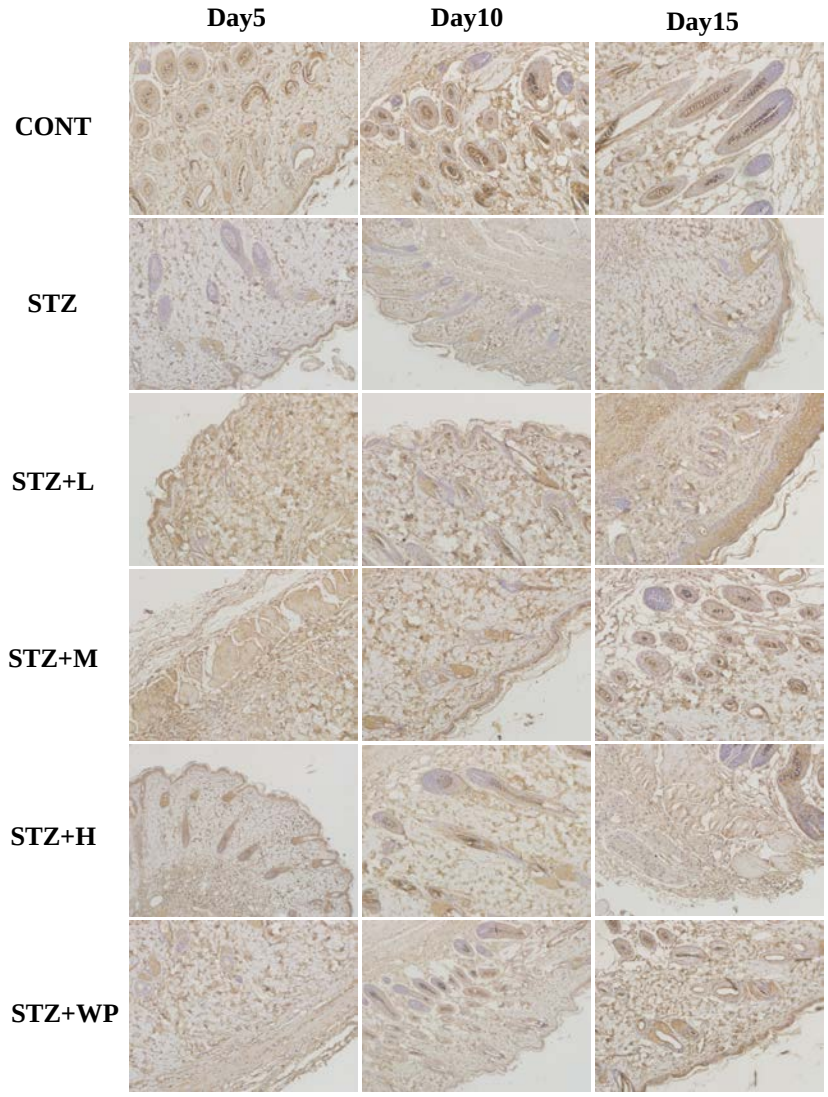


Fig. 3. Effects of TY001 on Insulin-like growth factor 1 (IGF-1) immunostaining in wound-surrounding skin tissues in STZ-induced diabetic mice, darker brown staining indicates more IGF-1 expression. Magnitude 100X.

### 3.6. TY001 enhanced basic fibroblast growth factor (FGF2) in the wound

To examine the mechanisms of promoted wound healing by TY001, the effects of TY001 on growth factors were examined. FGF2, also known as basic fibroblast growth factor and FGF-β, is a growth factor and signaling protein encoded by the FGF2 gene. Supplemental Figure 3 shows effects of TY001 on FGF2 immunostaining in wound-surrounding skin tissues in STZ-induced diabetic mice on day 15 of the treatment. The yellow-brown staining indicates the positive staining of FGF2. On day 5 of the treatment, the FGF2 staining in wound tissue of TY001 treated mice appeared and increased on day 10 and day 15 of treatment, much higher than STZ group.




Supplemental Figure 3. Representative photos of FGF2 immunostain. Arrows indicate more intense FGF2 immunostaing and higher FGF2 expression. magnitude 100X.

### 3.7. TY001 enhanced mRNA expression of growth factors in the wound

Fibroblasts are the main components of granules and secrete a lot of collagen, fibronectin, and extracellular matrix to provide microenvironment for skin epithelial cell regeneration. In

addition, fibroblasts secrete a lot of cytokines and growth factors, including Platelet-derived growth factor (PDGF); Transforming growth factor beta (TGF-β); Fibroblast growth factor (FGF); Vascular endothelial growth factor (VEGF); and Epidermal growth factor (EGF), for wound repair [1,2]. On days 5, 10, and day 15 of the treatment, the expression of these growth factors in wound tissues was significantly higher in TY001 group, in a dose-dependent manner. Compared to STZ only group, PDGF, TGF-β1, FGF, VEGF, and EGF expressions increased 3.6-, 3.9-, 4.4-, 5.1-, and 4.3-fold, in STZ+L, STZ+M, STZ+H, and STZ+WP groups, respectively at day 15 of the treatments (Figure 4).

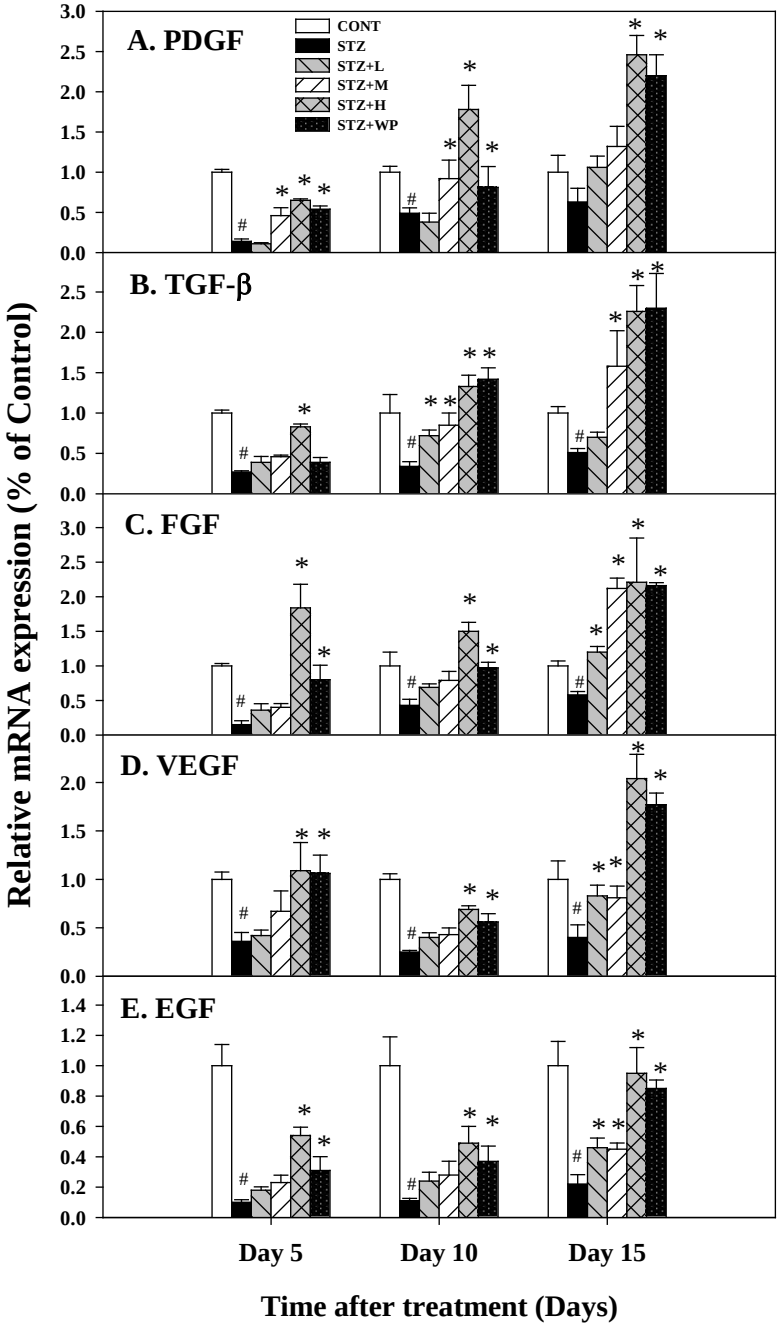


Figure 4. Effects of TY001 on growth factor mRNA expression. At 5, 10, and 15 days of treatments, the expressions of Platelet-derived growth factor (PDGF, A); Transforming growth factor beta (TGF-β, B); Fibroblast growth factor (FGF; C); Vascular endothelial growth factor (VEGF, D); and Epidermal growth factor (EGF, E) in wound-surrounding skin were. Data are mean ± SE of 6 mice. #Significantly different from controls, $p < 0.05$; *Significantly different between STZ and STZ+TY001, STZ+WP groups, $p < 0.05$.

### 3.8. TY001 improved serum cytokine and NO profiles and enhanced tissue SOD and CAT

Skin wound healing is a complicated physiological process involving tissue inflammation, regeneration and remodeling [7, 16, 17]. Inflammatory response is a key event in wound healing, removing contaminated bacteria to provide a suitable environment of tissue repair. On day 15 of the treatment, the serum cytokine levels of IL-1β (65%) and IL-8 (66%) were decreased by TY001 as compared to STZ diabetic mice, while the level of anti-inflammatory IL-10 was increased (224%) (Figure 5A, 5B, 5C). Serum nitric oxide (NO) levels were decreased 25% in the STZ groups, which was ameliorated by STZ+M (86% of Control), STZ+H (96% of Control), and STZ+WP (84% of Control) after 15 days of treatments (Figure 5F). At 5 and 10 days of treatments, TY001 and WP groups were also effective in ameliorating STZ decreased (Data not shown).

Increased reactive oxygen species (ROS) is a major factor delaying wound healing. ROS can be effectively scavenged first by superoxide dismutase (SOD), followed by catalase (CAT). Tissue SOD was decreased by 48% in STZ group, which was attenuated by STZ+L (92% of Control), STZ+M (74% of Control), STZ+H (93% of Control), and STZ+WP (102% of Control),

respectively (Figure 5D). CAT was also decreased 48% by STZ, which was recovered in STZ+M (76% of Control), STZ+H (88% of Control), and STZ+WP (88% of Control), respectively (Figure 5E).

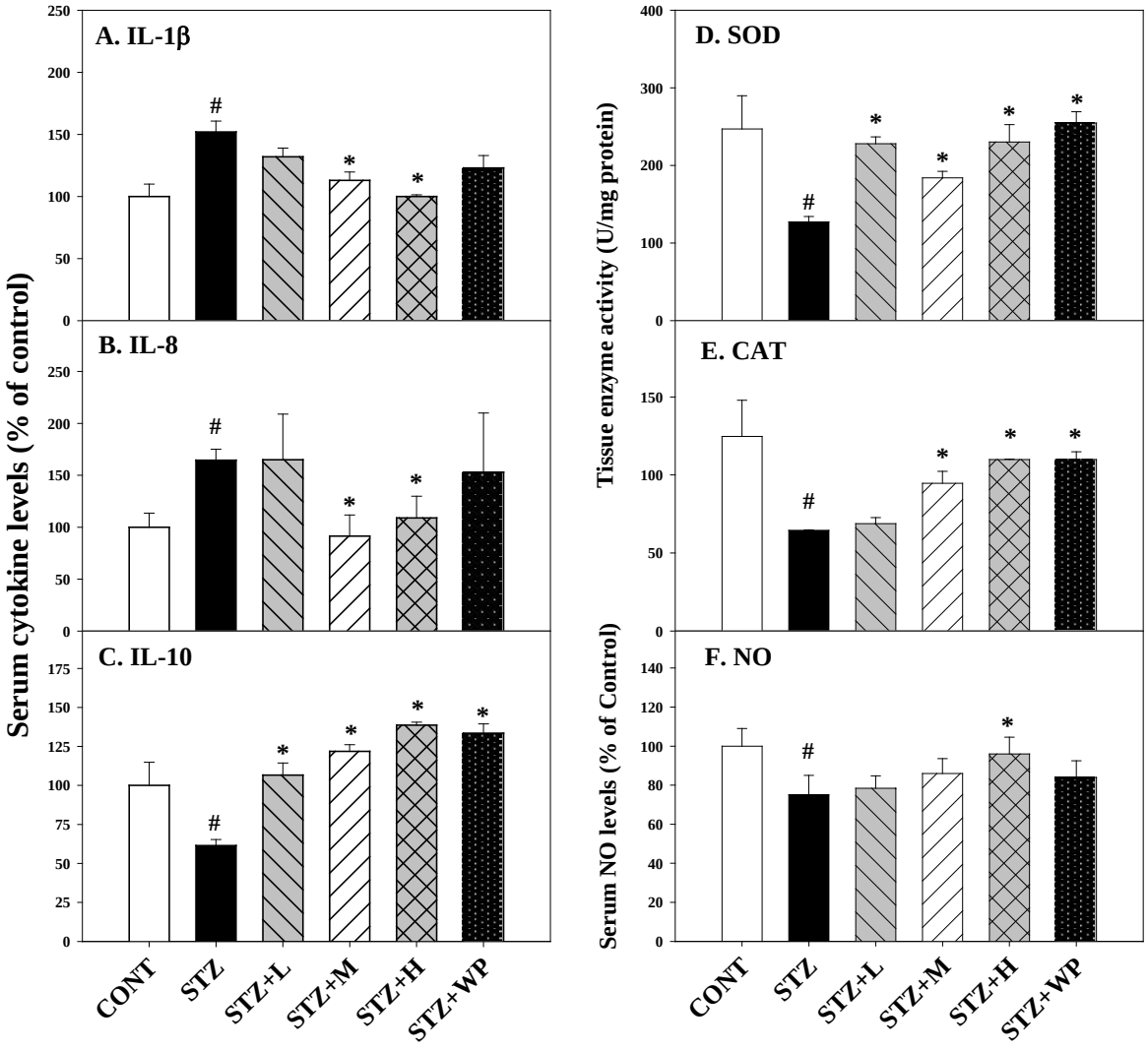


Fig. 5. TY001 improved serum cytokine and nitric oxide profiles and increased tissue antioxidant capacity. A, Serum interleukin 1β (IL-1β); B., Serum IL-8; C., Serum IL-10, D., Tissue superoxide dismutase (SOD); E, Tissue catalase (CAT), and F., Serum nitric oxide (NO) levels. Data are mean ± SE of 6 mice. #Significantly different from controls, $p < 0.05$; *Significantly different between STZ and STZ+TY001, STZ+WP groups, $p < 0.05$.

### 3.9. TY001 improved serum protein profiles

Sufficient nutritional support is important to correct stress and negative nitrogen balance, to provide essential nutrients for wound healing. Proteins are particularly needed to ensure tissue repair, and insufficient blood protein under diabetes conditions is one of the reasons for delayed wound healing. Indeed, serum levels of total protein, albumin, pre-albumin, and transferrin were decreased in diabetic mice, which were greatly increased with TY001 administration (Fig. 6).

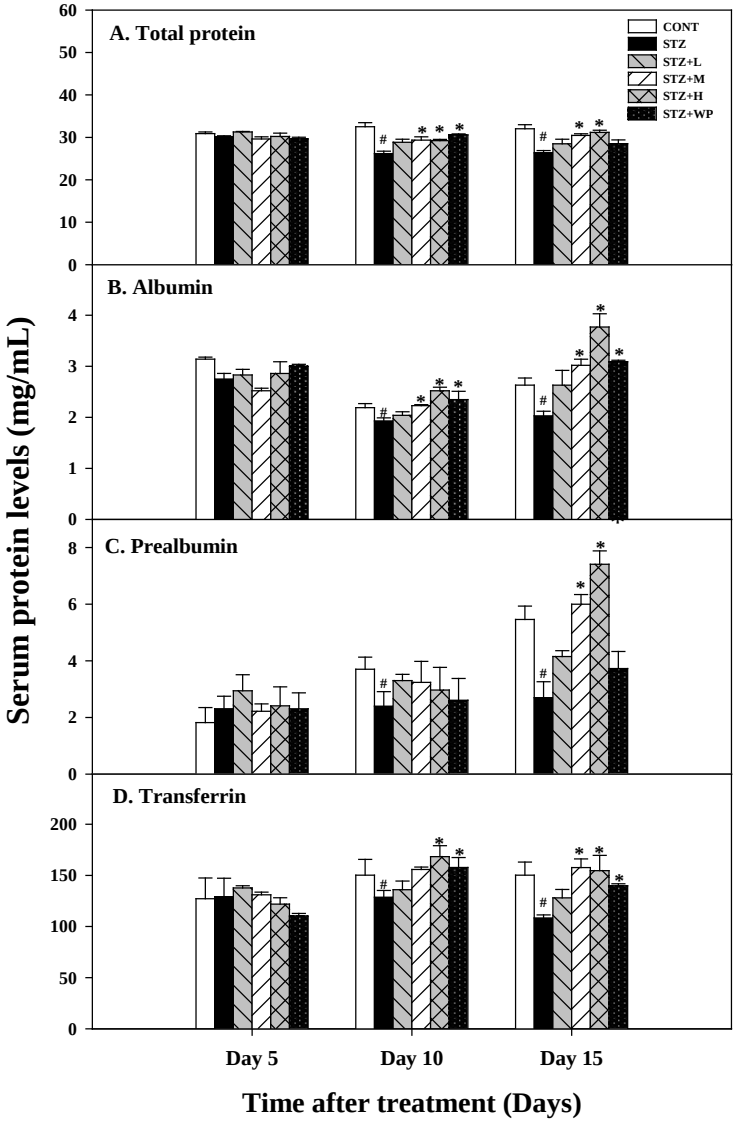



Fig. 6. TY001 improved serum protein levels. A., Serum Total protein; B., Serum Albumin, C., Serum prealbumin, and D., Serum transfection. Data are mean ± SE of 6 mice. #Significantly different from controls, $p< 0.05$; *Significantly different between STZ and STZ+TY001, STZ+WP groups, $p < 0.05$.

## 4. Discussion

TY001 is the unique Tilapia collagen peptides mixture, and it has been shown to be effective in promoting wound healing in acetic-induced Zebrafish skin lesions [14] and in protecting against LPS-induced inflammation and disruption of glucose metabolism and circadian clock [15]. The present study further demonstrated that TY001 was effective in wound healing in STZ diabetic mice, as evidenced by wound healing rates, decreased blood glucose levels in diabetic mice, and histopathology. The mechanisms of these beneficial effects of TY001 were related to enhanced expression of growth factors, reduced inflammation, and provided essential protein supplement in diabetic mice. TY001 was better than the known whey protein in diabetic wound healing.

TY001 is a collagen-peptide mixture, composed of whey protein concentrate, hydrolyzed wheat protein peptide, fish collagen peptide, calcium caseinate, wheat oligopeptide, and casein hydrolyzed peptide as described [14]. TY001 was composed by 47% of peptide with size lower than 1 KDa, 24% of peptide within 1-3 KDa, 10% of peptide within 3-5 KDa, and 12% of peptide over 5 KDa. [14], It has been shown that wheat peptides have antioxidant properties in reducing oxidative stress [18], and L-alaylglutamine from hydrolyzed wheat protein can be more efficiently absorbed than L-glutamine [19]. Collagen peptides can also be efficiently absorbed [20]. Casein hydrolyzed peptide could reduce stress in humans [21] and in animals [22], and have anti-inflammatory effects to promote wound healing [23]. These beneficial peptides in TY001 could act

in an integrated manner, rather by individual ingredient, to promote wound healing in STZ diabetic mice.

Histopathology clearly demonstrated that in STZ group, the wound surface on day 15 of the treatment had inflammatory cell infiltration, a lot of scar formation with less capillary formation. TY001 treatment apparently improved the pathological lesions, with reduced inflammatory cells and increased fibroblasts. Extracellular matrix deposition and remodeling are important events in wound healing, and reduced collagen deposition is characteristic in diabetes [24]. The increased collagen deposit was evidenced by Masson trichrome stain of the collagen fiber, but also by tissue content of hydroxyproline, an indirect indicator of collagen deposition [7, 16]. This study provided strong morphology evidence for TY001 in attenuating diabetic skin lesions and in promoting collagen deposition in diabetic mice.

Wound healing is regulated by growth factors and cytokines [25]. The current study revealed that IGF-1 and FGF2 were increased by immunohistochemistry at the wound-surrounding tissues, consistent with the literature [4, 25]. Angiogenesis was induced during healing process to maintain higher oxygen level to recruit cells to the wound spaces. Growth factors that associated with angiogenesis and wound regeneration have been identified, including PDGF [26], VEGF [5, 26], and TGF-β1 [5, 27]. Under diabetic conditions, TGF-β1, VEGF, and EGF expression are suppressed, impair the wound healing [5, 27]. In the present study, TY001 significantly increased the immunostaining of IGF-1 and FGF2, and increased the mRNA expression of

PDGF, TGF-β1, VEGF, and EGF, and higher expression of PDGF, TGF-β1, FGF and VEGF after TY001 than controls could be one of the mechanisms to overcome the wound healing delay under diabetic conditions. Overall, these data demonstrate that the activation of these growth factors is critical for wound healing in diabetic mice.

Inflammation is necessary to get rid of the dead cells for regeneration to occur. However, over inflammation under diabetic conditions will cause the delayed wound healing, because of increased ROS that would cause tissue damage [28]. Under diabetic conditions, prolonged inflammation could form a vicious cycle to delay wound healing [28-30]. Any means that could suppress inflammation through inhibition of NF-κB [17] or increase antioxidant capability are beneficial in accelerate diabetic wound healing [17, 31, 32]. Increased antioxidant capacity such as SOD, CAT through the Nrf2 activation pathways are thought to be a major component in promoting wound healing in STZ animals [31, 33]. The current study demonstrated that TY001 can reduce inflammatory cytokine production and to increase anti-inflammatory IL-10 levels, and increased tissue SOD and CAT activities to reduce prolonged inflammation to promote wound healing in diabetic mice.

Nitric oxide plays a central role in the regulation of three major parts of the wound healing process: vascular homeostasis, inflammation, and antimicrobial action [34]. Excessive NO, associated with infected or highly inflamed wounds, results in tissue damage during acute phase, while insufficient NO in diabetic wounds impedes migration and action of wound healing cell types during tissue regeneration pages. It has been shown that NO increases VEGF expression,

which is the most potent antigenic factor during wound healing, thereby stimulating the formation of new blood vessels [35]. In the present study, TY001 was effective in increasing STZ produced decrease in wound tissue during the tissue repair phase, which would help diabetic wound healing.

Diabetic patients have impaired immune function and malnutrition to delay wound healing [4, 25, 28, 31]. Dietary protein is a fundamental nutrient for animals and essential for organ physiological functions [36]. Nutrition supplement is important to provide essential amino acids and proteins for wound healing [7]. In the present study, serum total proteins, albumin, pre-albumin, transferrin these levels were all higher in TY001 groups, suggesting that TY001 supplement improved nutritional status in diabetic mice.

The microbiota plays an important role in many metabolic functions, including glucose and lipid homeostasis, and altered microbiota composition is found to be associated with diabetes, host immune function, inflammation and many other diseases [37, 38]. Many dietary supplements could be beneficial by modulating gut microbiota to exert biological effects. For example, dietary grape seed proanthocyanidins [39], dietary sodium butyrate [40], dietary lipoic acid [41], dietary dimethyl fumarate [42], and moderate dietary protein restriction [36] could modulate intestinal permeability and gut microbiota composition to promote animal growth and to reduce diarrhea [38]. It should also be noted that inflammation is closely associated with host-microbiota interaction [38, 43, 44]. In patients with inflammatory bowel disease, altered gut microbiota is associated with disease development and progression [45, 46]. Thus, dietary supplements are becoming novel

therapeutic approaches [38, 47]. Whether oral TY001 through the drinking water could have any effects on intestinal integrity and gut microbiota to exert anti-inflammatory effects and promote diabetes wound healing warrants further investigation.

## 5. Conclusions

This study clearly demonstrated that the collage mixture TY001 was effective in promoting wound healing in STZ diabetic mice. The mechanisms appeared to be improved glucose metabolism, increased growth factor expression, anti-inflammation, and nutritional supplement. Diabetic wound healing is a much-needed therapy and this novel collagen-peptide mixture could be a promising candidate in clinical application in the promotion of wound healing in diabetic patients.

**Conflicts of Interest:** The authors declare no conflict of interest.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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