Effect of Aloe Vera on MMP-1 and TIMP-1 Expression on Diabetic Wound Healing

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Abstract
Aim: The aim of this study is to investigate the healing aspect of aloe vera in diabetes mellitus, which inhibits wound healing.
Methods: Diabetes model was created with streptozotocin. At the end of the 14-day experiment, blood glucose was measured from the tail vein of animals in all groups and blood was taken from the heart and sacrificed. Histopathology and immunohistochemical statistics and evaluation were performed.
Results: Pycnosis and degeneration of epithelial cells were observed in diabetes groups. Leukocyte infiltration in the dermal papilla, degeneration of collagen fibers and an increase in the extracellular matrix were observed. It was observed that the epithelial layer in the aloe vera group was histologically close to the control group. It was observed that decreased inflammation in the dermal papilla and decreased in organized collagen fibers and vessel dilatation were observed. In the control group, MMP-1 and TIMP-1 expression were positive in the epidermis and dermis layers. In the diabetes group, weak expression of MMP-1 and TIMP-1 was observed in cells in the epidermis and dermis. The expression of MMP-1 and TIMP-1 in the surface epithelium in the aloe vera group was increased compared to the diabetes group.
Conclusions: Aloe vera accelerated cell and extracellular matrix regeneration with its anti-oxidative activity.
Keywords: *Diabetes, wound healing, MMP, Aloe Vera, TIMP-1*

1. Introduction

Diabetes Mellitus is a lifelong disease that develops when the pancreatic gland does not produce enough insulin hormone or the insulin hormone it produces is not used effectively. Diabetes is responsible for the delayed or incomplete wound healing process seen in patients and often leads to the formation of chronic ulcers[1]. Matrix metalloproteinases (MMPs) cleave the peptide bonds of extracellular matrix proteins such as collagen, laminin, elastin and fibronectin. This family of proteinases plays a critical role in morphogenesis, development, wound healing, reproduction, and neovascularization. Also, this proteolytic enzyme activity is controlled by another family of proteins, tissue metalloproteinase inhibitors (TIMPs). The imbalance between MMPs and TIMPs plays a role in many pathological processes such as cancer metastasis, arthritis, inflammation, periodontal diseases, corneal ulceration and cardiovascular diseases. In the skin, this enzyme is synthesized by fibroblasts and keratinocytes and plays an important role in the amount of dermal collagen in the tissue[2]. In a previous study evaluating wound healing in humans, it was shown by protein analysis that the balance between MMP-1 and TIMP-1 plays an important role[3]. Many clinical and experimental studies examining the effects of herbal extracts on acute or pathological wound healing have been conducted and discussed. Aloe Vera produces gel and latex, two substances used to make medicine. Aloe Vera gel is a clear, jelly-like substance found on the inside of the Aloe plant leaf[4]. Aloe latex is a yellow substance located just below the plant membrane. Studies have shown that Aloe Vera moisturizes the skin, is good for sunburn and skin wrinkles, helps to maintain the elasticity and freshness of the skin, and to control acne and eczema. In addition, the healing power of Aloe Vera on the skin is due to the increase in the amount of oxygen and collagen synthesis in the skin[5]. In this study, the effectiveness of aloe vera on the balance of MMP-1 and TIMP-1 in rats with experimental diabetic wound model was investigated by histopathological and immunohistochemical methods.

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Received: 21.03.2023, Accepted: 24.05.2023, Available Online Date: 31.08.2023
Cite this article as: Seyrek R, Soker S, Kaplan O, et al. Effect of Aloe Vera on Mmp-1 and Timp-1 Expression on Diabetic Wound Healing. J Cukurova Anesth Surg. 2023; 6(1): 204-9. doi: 10.36516/jocass.1268067

2. Materials and methods

This study was carried out within the framework of the permission dated 29.04.2020 and numbered 2020/09, obtained from the Dicle University Animal Experiments Local Ethics Committee. In our study, 21 Wistar Albino female rats, 15-16 weeks old and weighing between 190-210 g, were used. Rats were divided into 3 groups.

1. Sham group: A wound model with a diameter of 1 cm was created on the dorsal skin in the lumbosacral vertebral region of the animals.

2. DM group: STZ-induced diabetes was induced by intraperitoneal administration of 45 mg/kg streptozotocin (STZ) dissolved in cold 0.1 M citrate buffer (pH=6.0).72 hours later, those with a glucose level > 240 mg/dl measured with a glucometer from the tail vein were considered Type 2 diabetic[6].A wound model with a diameter of 1 cm was created on the dorsal skin in the lumbosacral vertebral region of the animals.

3. DM+Aloe vera group: STZ-induced diabetes was induced by intraperitoneal administration of 45 mg/kg streptozotocin (STZ) dissolved in cold 0.1 M citrate buffer (pH=6.0).72 hours later, glucose levels > 240 mg/dl measured with a glucometer from the tail vein were considered Type 2 diabetic. Aloe vera extract was dissolved in distilled water and 300 mg/kg daily was given as 0.5 ml suspension by oral gavage. A wound model with a diameter of 1 cm was created on the dorsal skin in the lumbosacral vertebral region of the animals.

At the end of 14 days, blood glucose was measured from the tail vein and blood was taken from the heart and sacrificed. Tissues were excised and removed and skin samples were fixed in 10% formol. Skin samples were dehydrated and incubated in xylene. The tissue samples were then embedded in paraffin blocks. Skin tissue sections taken from paraffin blocks were stained with Hematoxylin-Eosin (HE), Alcian Blue and immunohistochemistry[7].

2.1. Immunohistochemical staining

Sections were deparaffinized in xylene for 3x15 minutes. Sections were passed through the decreasing alcohol series for 10 minutes and put into water. Sections were washed 3x5 minutes in phosphate buffer solution (PBS). Sections were taken in ethylenediamine tetraacetic acid (EDTA) buffer solution (pH:8.0, catalog no: ab93680, Abcam, Cambridge, USA) and retrieval of the heat-induced epitope was performed. Hydrogen peroxide solution (catalog no: TA-015-HP, ThermoFischer, Fremont, CA, USA) was dripped onto the sections and left for 20 minutes. It was kept in Ultra V Block (catalog no: TA-015-UB, ThermoFischer, Fremont, CA, USA) solution for 7 minutes. Sections were kept at +4°C overnight with antibodies to MMP-1 (catalog no:bs-0463R) and TIMP-1 (catalog no:orb-195994). Biotin-containing secondary antibody (catalog no: TP-015-BN, ThermoFischer, Fremont, CA, USA) was dripped onto the sections and left for 14 minutes. Then, streptavidin-peroxidase (catalog no: TS-015-HR, ThermoFischer, Fremont, CA, USA) was dripped and left for 15 minutes. Diaminobenzidine (DAB) (catalog no: TA-001-HCX, ThermoFischer, Fremont, CA, USA) was dropped on the sections washed with PBS. After counterstaining with Harris hematoxylin, the sections were covered with entellan (catalog no:107961, Sigma-Aldrich, St. Louis, MO, United States). They were evaluated and visualized under a Zeiss Imager A2 photomicroscope.

2.2 Statistical Analysis:

IBM SPSS version 25 software program was used for statistical analysis. Multiple comparisons between groups before and after the experiment were made according to Kruskal Wallis and post hoc Tamhane's T2 test. $P<0.05$ was considered statistically significant and the results were shown as mean ± standard deviation (SD).

3. Results

3.1. Blood Glucose Level

Numerical and graphical data on blood glucose levels are shown in Table 1 and Figure 1. Pre-experimental blood glucose levels were 110.71 ± 11.29 mg/dl in the sham group, 118.00 ± 25.51 mg/dl in the diabetes group, and 119.57 ± 16.97 mg/dl in the diabetes+aloe vera group. and no statistically significant difference was found between the groups ($p>0.05$). At the end of the experiment, the blood glucose level of the diabetes group was found to be 367.86 ± 113.55 mg/dl and significantly higher than the sham group ($p<0.01$). It was determined that the blood glucose level of the diabetes+aloe vera group was 220.86 ± 5.49 mg/dl and was significantly different from the sham group ($p<0.01$) and diabetes group ($p<0.05$).

3.2. Histopathological Findings

Hematoxylin staining (a-c) and alcian blue (d-f) stainings of Sham, diabetes and aloe vera groups are shown in Figure 2.

Sham Group: Cells were observed in the epidermis layer with normal appearance. A tight connective tissue and hair follicle structures were observed in the dermis region. Vascular structures were observed regularly in the dermis (Figure 2a).

Diabetes Group: Degeneration loss in epithelial cells and pycnosis in nuclei were observed. Thinning and separations were detected in the epidermis structure. Leukocyte infiltration was observed in the dermal papilla, slight degenerations were observed in collagen fibers and fibrosis began. Degenerative changes were observed in hair follicles. Apoptotic changes were detected in the nuclei of connective tissue cells (Figure 2b).

Aloe Vera Group: Thickness was detected in the epidermis layer. Collagens were detected regularly in the dermis layer. Mild hypertrophy was observed in connective tissue cells, vessels were dilated and mild leukocyte infiltration was detected (Figure 2c).

Control Group: Epidermis layer was observed regularly. It was observed that there was an irregular tight connective tissue between the collagen fibers rich in dermal papilla ESM.

Table 1

Blood glucose levels before and at the end of the experiment

	Sham	Diabetes	Diabetes+Aloe vera
Pre Experiment	110,71 ± 11,29a	118,00 ± 25,51a	119,57 ± 16,97a
Post experiment	107,57 ±7,28a	367,86 ± 113,55c	220,86 ± 5,49b

Note :Different superscripts indicate significant difference between groups: a-bp<0.01, a-cp<0.01, b-cp<0.05.

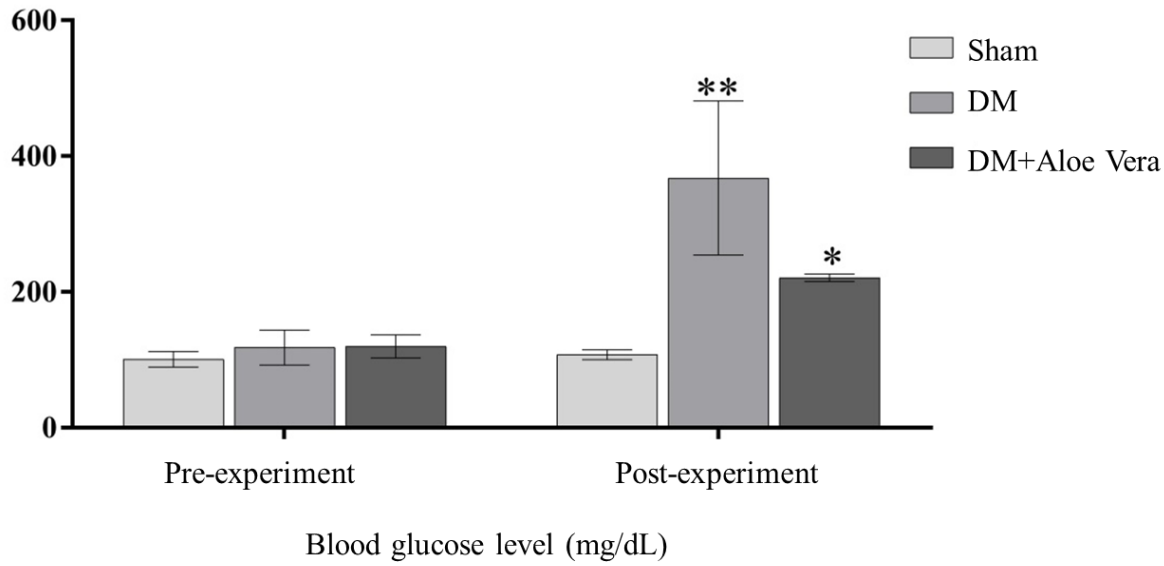

Figure 1

Graphical presentation of the statistical analysis of the Pre-Experimental and Pre-Sacrification Blood Glucose Levels of the Groups. According to Sham group *p<0.01, **p<0.01. */**p<0.05.

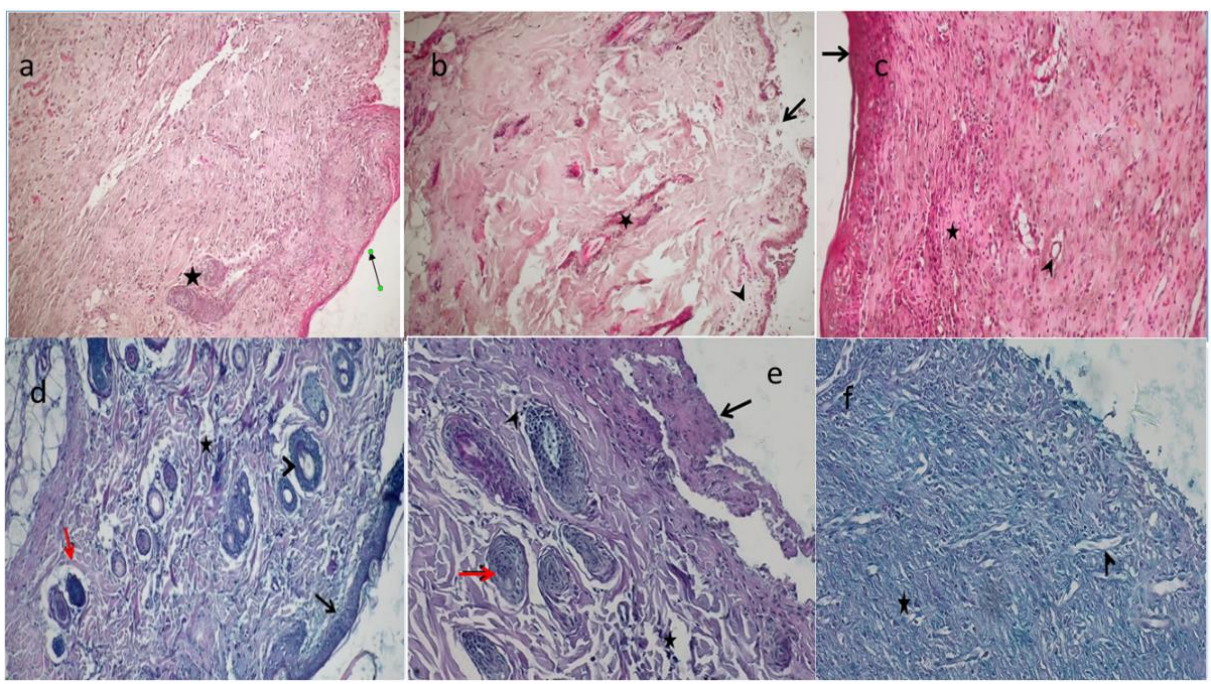

Figure 2

a) Control Group: Epidermis (arrow) follicle (star), b) Diabetes group: Epidermis (arrow) leukocyte infiltration (arrowhead), collagen structure (star); c) Aloe vera group: Epidermis (arrow), fibroblasts (star), vascular structure (arrowhead) Bar: 100μm Hematoxylin eosin 10X d) Control Group: Epidermis (arrow), collagen fiber (star), hair follicle (arrowhead), musculus erector pili muscle (red arrow); e) Diabetes Group: Epithelium (arrow), leukocyte cells (arrowhead), hair follicle (red arrow), collagen fibers (star). f) Aloe Vera Group: Collagen fibers (star), vessel dilatation (arrowhead) Bar: 100μm Alcian Blue 10X

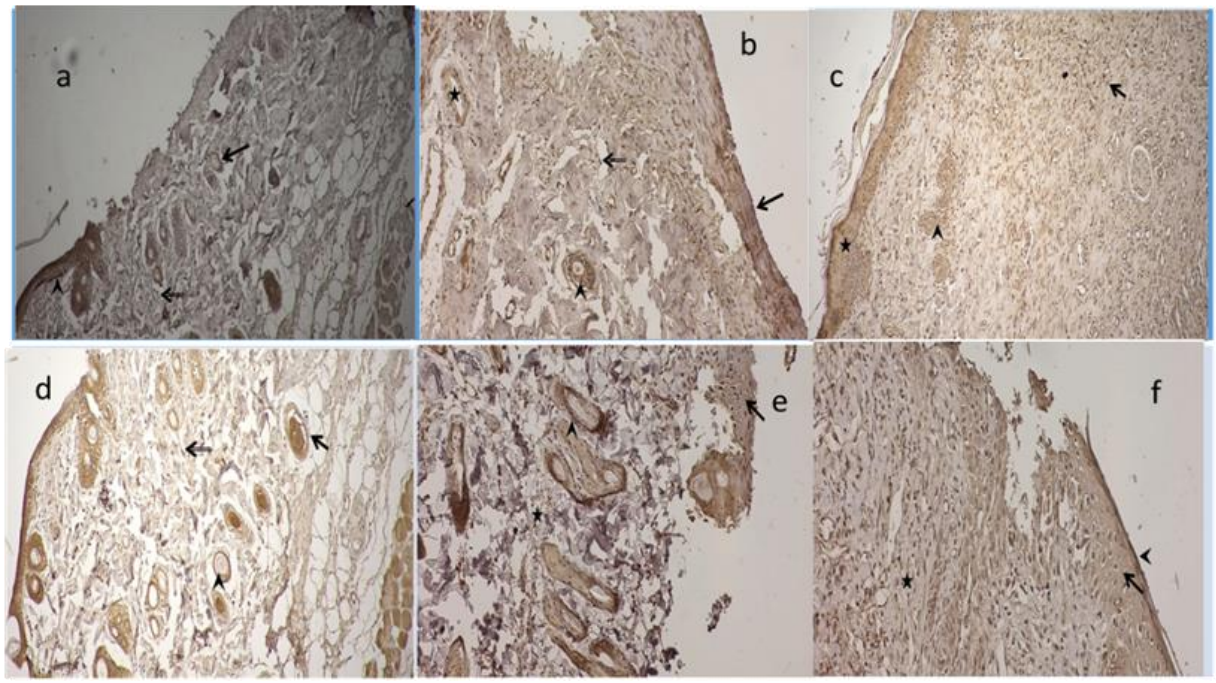

Figure 3

a) Control Group: Basal lamina (arrowhead) in the epidermis, fibroblast (double-tailed arrow) and in hair follicles (arrows) positive MMP-1 expression (arrows) b) Diabetes Group: Epidermis (arrow), in the basal lamina of the vessels (star), hair follicles (arrowhead), fibroblast cells (double-tailed arrow) MMP-1 positive expression. c)Aloe Vera Group: Granulosa cells (star), hair follicle (arrowhead), fibroblast cells (arrow) positive MMP expression. d) Control Group: in hair follicles (arrow), in Huxley layer (arrowhead) and fibroblasts (double-tailed arrow) positive TIMP-1 expression; e) Diabetes Group: Positive TIMP-1 expression in granulosa cells (arrow), connective tissue (star), hair follicles f) Aloe Vera Group: It was determined that TIMP-1 reaction was intense in the epidermis (arrow), in keratinized areas (arrowhead), fibroblasts and other connective tissue cells (star). Bar: 100μm 10X. Immunohistochemical Staining

The outer hair follicles were seen in normal appearance. The musculus erector pili muscle was located parallel to the hair follicle (Figure 2d).

Diabetes Group: Epithelial loss was observed. Leukocyte cells were detected in the dermis layer. Apoptotic changes were observed in the hair follicle. Degenerative changes were seen in collagen fibers, an increase in ESM structure was observed. The vessels are slightly dilated (Figure 2e).

Aloe Vera Group: A significant decrease was observed in the connective tissue between the collagen fibers in the dermis. Connective tissue cells were prominent in the dermis, with a marked reduction in dilatation and inflammation (Figure 2f).

3.3 Immunohistochemical Findings

Figure 3 shows MMP1 (a-c) and TIMP1 (d-f) stainings of Sham, diabetes and aloe vera groups. Control Group: MMP-1 expression in the basal lamina region of the epidermis, hair follicles and fibroblast cells was evaluated as positive (Figure 3a). Diabetes Group: It was determined that MMP-1 expression was positively stained in epidermis cells, hair follicles, and fibroblast cells (Figure 3b). Aloe vera group, It showed a very intense increase in MMP-1 expression in the surface epithelium, hair follicle and fibroblast cells (Figure 3c). Control Group: TIMP-1 expression intensity was observed in hair follicles, Huxley layer, fibroblasts in the dermal papilla region (Figure 3d). Diabetes Group: TIMP1 expression was positive in the epidermis, connective tissue cells and hair follicles (Figure 3e). Aloe vera Group: TIMP-1 reaction was found to be positive in the epidermis, fibroblasts and other connective tissue cells (Figure 3f).

4. Discussion

Wound healing is a natural process involving complex cellular and biomolecular steps that are shaped to restore the tissue to its former state after damage. Basically, the biological wound healing process takes place through the regulation of homeostasis, inflammation, cell migration and proliferation, and remodeling. Proper wound healing leads to rapid wound closure and minimal or aesthetically acceptable scarring without regeneration for the acute wound [However, while this process occurs at an optimal rate and in an optimal manner in healthy individuals, in people with diabetes, wound healing is impaired or disrupted[8].

DM is a metabolic disease characterized by high blood glucose levels caused by a decrease in insulin secretion and/or a decrease in the effect of insulin. Various factors such as environmental, genetic and lifestyle factors have been reported to contribute to the development of DM[9]. DM, which is a progressive disease, becomes a major health problem for society and the individual, leading to increased morbidity and mortality with serious complications if not controlled[5].

Many factors that inhibit the wound healing process at various stages lead to delayed wound healing and an increase in mortality and morbidity[10]. Although the adverse effects of DM on wound healing have not been fully explained, high blood glucose levels are thought to be the underlying cause of this condition by inhibiting cell proliferation and collagen production, decreasing fibroblast formation and growth factors, increasing apoptosis in wound tissue cells, increasing infection formation due to decreased angiogenesis,

granulation tissue formation, chemotaxis and phagocytosis[11]. DeClue and Shornicks reported that diabetic wound healing is associated with excessive release of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α[12]. Qui et al. reported that diabetic patients with high blood glucose levels had decreased cell proliferation and decreased collagen production and growth factors in the wound healing process[13]. Decreased angiogenesis and decreased growth factors such as VEGF and TGF-1β are thought to be associated with the non-healing process of diabetic wounds[14].

The use of medicinal plants for healthy living, treatment and care of various diseases has increased rapidly worldwide in recent years. 60% of the world population and 60-90% of developing countries use traditional medicinal plants in primary health care[15]. However, one out of every three drugs used in traditional medicine is used in wound and skin diseases, while only 1-3% of synthetic drugs are used in these diseases[16]. All these suggest that medicinal plants used in wound treatment may have the potential to be therapeutic alternatives to synthetic drugs.

Aloe vera (yellow patience) belonging to the Liliaceae family is known to have wound healing properties and has been used for thousands of years for this purpose[17, 18]. Atiba et al. examined the effects of oral Aloe vera application on diabetic wounds in a study on type II diabetic rat models and reported that this application increased inflammatory cell infiltration, angiogenesis, extracellular matrix deposition and epithelialization and accelerated wound contraction, and also increased TGFβ-1 and VEGF protein-positive cells[19]. In another study, the positive effects of oral and topical Aloe vera application on the wound were shown on diabetic rat models[20]. In the rats treated with Aloe vera, the wound patency level was visibly between the levels in the sham and diabetes groups. In microscopic examination, macrophage and lymphocyte infiltration was quite intense in the wound tissue in the sham group and regeneration of epithelial and connective tissue elements was detected. In the diabetes group, the epidermis layer could not reach a compact state, intense edema accumulated in the wound area and the general structure of the scar tissue was observed. In the diabetes group of our study, loss of epithelial cells in the epidermis, pyknotic leukocyte infiltration in the nucleus and solitary leukocyte infiltration in the dermal papilla region, degeneration of collagen fibers, and cell disruption and apoptotic changes in the hair follicles were observed (Figure 2b,2d). Although cell nucleus shrinkage was observed due to the effect of diabetes, degenerative changes increased and apoptotic changes accelerated. In the group treated with aloe vera, epithelium size and nucleus size were close to the sham group and regular, inflammation in the dermal papillae decreased, collagen fibers were regular, vascular dilatation decreased and hair follicles started to improve. It was thought that the tightening property of aloe vera especially in the dermis region was important in terms of collagen reorganization (Figure 2c,2f).

It is known that the amount of MMP in the tissue decreases over time in the normal wound healing process, but in chronic wounds, there is an increase in proteases as well as proinflammatory cytokines and the amount of growth factors decreases[21-23]. Lobmann et al. In their study, Lobmann et al. reported that the expression level of MMP-1, MMP-8 and MMP-9 and the level of active MMP-2 increased significantly in diabetic ulcers, whereas the level of TIMP-2, the tissue inhibitor of MMP-2, decreased. Muller et al. examined the levels of various MMP and TIMP-1 in cutaneous tissue fluid in diabetic patients with foot ulcers. As a result of their study, they reported that the amount of MMP-1 and TIMP-1 in the tissue fluid of patients with good wound healing increased by the 2nd week, but both MMP-1 and TIMP-1 levels tended to decrease in the following weeks. The researchers stated that in patients in whom wound healing did not progress normally, the TIMP-1 level remained almost constant during the 12-week study, but after the 12th week, there was an increase in MMP-1 level and a decrease in TIMP-1 level. The researchers reported that MMP-1 and TIMP-1 play an active role in the wound healing process and that an increase in both the amount of MMP-1 and the MMP-1/TIMP-1 ratio may provide information about the healing of diabetic ulcers in patients whose wound healing process is clinically well evaluated[24].

In our study, when the cutaneous wounds of animals with experimental diabetes model were compared with the cutaneous wounds of non-diabetic animals at the end of the 14-day follow-up, it was found that MMP-1 immunoexpression was significantly increased and TIMP-1 expression was decreased. In animals in which we administered 300 mg/kg Aloe vera oral gavage for 14 days, it was observed that both MMP-1 and TIMP-1 expression levels were at a level between the sham and diabetes groups. In the sham group, MMP-1 and TIMP-1 expression in the epidermis and dermis, especially in the regions where extracellular matrix was dense and in the external membrane of the hair follicle was prominent (Figure 3a,3d). In our study, it was found that both cellular and fibrous structuring decreased in both epidermis and dermis in diabetes-induced wound healing, and the matrix metalloprotein family showed a significant decrease in membranes in terms of both hair follicle and collagen fibers in both dermis and epidermis. Weak MMP-1 and TIMP-1 expression was observed in epidermis and dermis cells in the diabetes group (Figure 3b,3e). Although topical application of aloe vera is widely used under normal conditions, in our study, it was observed that the plant spread over a certain time interval in wound healing as a result of oral administration, but regional diffusion was slightly delayed. It was thought that the delay in wound healing with diabetes may be related to the inhibition of diabetes in two different regions in both epidermis and dermis. Although the effect of aloe vera has been shown in many studies, it was understood that it induced the reorganization of cells in the epidermis and the extracellular matrix and collagen fiber structuring model in the dermis. The fact that the extracellular matrix density in the Aloe vera group appeared close to the sham group, especially in terms of MMP-1 and TIMP-1, was considered as a signal of the connective tissue reorganization process. The increase in MMP-1 and TIMP-1 expression in the aloe vera group (Figure 3c,3f) was shown to be an important feature of tightening and repair in the dermis.

5. Conclusions

As a result of the anti-oxidative effect of aloe vera, it has been observed that the restructuring of cells and the development of extracellular matrix organization, depending on the decrease in oxidative stress, accelerated and the effect on skin tightening began to increase. Aloe vera administration alleviated pathology and promoted cell proliferation induced by diabetes with its anti-oxidative activity.

Acknowledgements

None.

Statement of ethics

This study was approved by Dicle University Faculty of Medicine Ethics Committee for Animal Experimentation with the protocol number (2020/09).

Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Funding source

This study was supported by Dicle University Scientific Re-

search Projects Commission with project number TIP.20.027

Author contributions

All authors contributed to the study conception and design.

All authors read and approved the final manuscript.

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