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Research Article

Does Low Dose N-Acetylcysteine Administration Enhance Diabetic Wound Healing?

Emine Gülçeri GÜLEÇ PEKER[1*]

[1] Faculty of Health Sciences, Giresun University, Giresun, Turkey

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Abstract
Oxidative stress and persistent inflammation play crucial role in the progression of diabetic wound complications. N-Acetylcysteine (NAC) is an amino acid derivative that promotes wound healing because of its ability of antioxidant properties. The aim of this study was to evaluate the effect of systemic NAC administration on oxidative stress, inflammatory markers, and collagen production in diabetic wound healing. Induction of diabetes was done by intraperitoneally administration of streptozotocin in Wistar rats. A dorsal circular wound was created in diabetic rats. After that subjects were treated with NAC (60 mg/kg) intraperitoneally to evaluate the wound healing potential of NAC. In the wound tissues of diabetic rats, lipid peroxidation and inflammatory markers were significantly increased (p<0.05). Superoxide dismutase and catalase activities, glutathione and NOx levels, and collagen production were significantly reduced in diabetic group (p<0.05). NAC administration increased antioxidant status and NOx levels, reduced lipid peroxidation and inflammatory marker levels in treated diabetic group compared to diabetic group (p<0.05). Additionally, in the diabetic NAC treatment group, collagen production was significantly increased and wound area was significantly smaller than diabetic group (p<0.05). Low dose NAC administration was found to be effective in diabetic wound healing by reducing oxidative stress and inflammation and increasing collagen production.
Keywords: Wound healing, diabetes, N-acetylcysteine, oxidative stress, pro-inflammatory markers

Düşük Dozda N-Asetilsistein Uygulaması Diyabetik Yara İyileşmesini Hızlandırır mı?

Öz
Oksidatif stres ve kalıcı inflamasyon, diyabetik yara komplikasyonlarının gelişmesinde önemli rol oynar. N-Asetilsistein (NAC), antioksidan özelliklerinden dolayı yara iyileşmesini hızlandıran bir amino asit türevidir. Bu çalışmanın amacı, sistemik NAC uygulamasının diyabetik yara iyileşmesinde oksidatif stres, inflamatuar belirteçler ve kollajen üretimi üzerindeki etkisini değerlendirmektir. Wistar sıçanlarda diyabet indüksiyonu, streptozotosinin intraperitoneal olarak uygulanmasıyla yapılmıştır. Diyabetik sıçanlarda dorsal dairesel bir yara oluşturulmuştur. NAC'nin yara iyileştirme potansiyelini değerlendirmek için denekler intraperitoneal olarak NAC (60 mg / kg) ile tedavi edilmiştir. Diyabetik sıçanların yara dokularında lipid peroksidasyonu ve inflamatuar belirteçler önemli ölçüde artmıştır (p<0.05). Diyabetik grupta süperoksit dismutaz ve katalaz aktiviteleri, glutatyon ve NOx seviyeleri ve kollajen üretimi anlamlı olarak azalmıştır (p<0.05). NAC uygulaması, diyabetik gruba kıyasla, tedavi edilen diyabetik grupta antioksidan durumu ve NOx düzeylerini arttırmış, lipid peroksidasyonunu ve inflamatuar belirteç düzeylerini düşürmüştür (p<0.05). Ayrıca diyabetik NAC tedavi grubunda kollajen üretimi anlamlı olarak artmış ve yara alanı diyabetik gruba göre anlamlı olarak küçülmüştür (p<0.05). Düşük doz NAC uygulamasının, oksidatif stresi ve inflamasyonu azaltarak ve kollajen üretimini artırarak diyabetik yara iyileşmesinde etkili olduğu bulunmuştur.
Anahtar Kelimeler: Yara iyileşmesi, diyabet, N-asetilsistein, oksidatif stres, pro-inflamatuar markörler.

1. Introduction

Delayed or impaired wound healing is one of the most significant complications of diabetes, with diabetic foot ulcers (DFU) often seen clinically. Serious ulcerations can result in limb amputation (Westyb et al., 2020), which adversely affects the patient's

*Corresponding Author: gulceri.peker@giresun.edu.tr

quality of life. Normal wound healing is an integrated and highly complex series of events that require the interaction of many cell species such as keratinocytes, endothelial cells, inflammatory cells, and fibroblasts, accompanied by the action of growth factors and enzymes. The wound healing process consists of several successive steps: inflammation, re-epithelialization, neovascularization, granulation tissue formation, contraction, scar tissue formation, and tissue remodeling (Brocke and Barr, 2020). The inflammatory phase, the first stage in wound healing, is a critical step to prevent the development of infection and initiate tissue regeneration. Diabetic wound healing is characterized by a prolonged inflammatory phase caused by an increase in inflammatory cytokines, delayed re-epithelialization, and oxidative stress due to the overproduction of reactive oxygen species (ROS) (denDekker et al., 2019). Hyperglycemia contributes to this delayed/impaired healing process in diabetes, leading to increased polyol pathway activity in cells. Increased conversion to fructose leads to a decrease in nicotinamide adenine dinucleotide phosphate (NADPH) and an increased risk of oxidative stress due to elevated superoxide radical (O_2^-) production. In the presence of hyperglycemia, advanced glycation end products (AGE) are formed as a result of the non-enzymatic glycation of collagen and other proteins. AGE can cause increased oxidative stress and activate nuclear factor kappa B (NF-κB), which is an important transcription factor that leads to inflammation and decreased resolution of the extracellular matrix (Boniakowski et al., 2017). There is a sensitive balance between the levels of ROS and antioxidants to prevent oxidative damage in biological systems, which changes in favor of ROS in diabetes. ROS and antioxidant systems that eliminate free radicals play a key role in normal and diabetic wound healing (Dunnil et al., 2017; Fui et al., 2019). ROS such as O_2^-, hydrogen peroxide (H_2O_2), and nitric oxide (NO) act as cellular messengers to stimulate phases of wound healing and defend against microorganisms that invade the wound site. On the other hand, endogenous enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), in addition to endogenous non-enzymatic antioxidants such as glutathione (GSH), prevent the overproduction of ROS during the early stages of wound healing. When antioxidative defense systems cannot adequately scavenge ROS, oxygen radicals damage cell lipids, proteins, and DNA, causing oxidative stress (Tort et al., 2020; Thi et al., 2020). N-acetylcysteine (NAC) is a thiol compound clinically approved for use as a mucolytic agent (Aldini et al., 2018). The effects of NAC, such as protein kinase B phosphorylation, NF-κB activation, and reduction of oxidative stress and inflammation, have been shown to occur by different mechanisms including translocation of cytosolic proteins (Wang et al., 2013). NAC activates endothelial cells (EC) through mechanisms that cannot yet be fully explained. Moreover, it has been reported that NAC may have a wider therapeutic potential, especially in diabetes (Lasram et al., 2015). In rodent diabetic models, NAC has been shown to increase insulin sensitivity (Ismael et al., 2008; Lasram et al., 2015). Several clinical and experimental studies have indicated beneficial effects of NAC on insulin resistance and type 2 diabetes (Shen et al., 2018; Li et al., 2020). Positive contributions of NAC treatment to wound healing have also been demonstrated (AlMatar et al., 2016; Adil et al., 2018; Janeczek et al., 2019). Therapeutic dosages

of NAC have not been conclusively specified in the literature; thus, further studies are needed to determine optimal therapeutic dosing. Research related to the effects of NAC on diabetic wound healing *in vivo* are limited. Accordingly, the present study aimed to investigate the effects of systemic administration of low-dose NAC (60 mg/kg) on wound healing and oxidative parameters in rats with streptozotocin (STZ)-induced diabetes.

2. Material and Methods

Twenty-four (24) adult male Wistar albino rats weighing 250–300 g was used in the present study. The animals were allowed to acclimatize to the laboratory conditions for a week before beginning the experiment. Prior to and during the experiments, all rats were placed in separate cages in a light- and temperature-controlled environment (12/12 h light/dark cycle and 25 ± 3°C). Animals had access to water and standard food *ad libitum*.

Ethical statement

All experimental procedures performed in animals were approved by the Giresun University Animal Experiments Ethics Committee (12763492-600-E.62401). All animal experiments were carried out in the Giresun University Experimental Animals Laboratory.

Diabetic model

To create the diabetic model, single-dose intraperitoneally (IP) STZ injection (60 mg/kg body weight) dissolved in sodium citrate solution (0.1 molar, pH 4.5) was administered to overnight-fasted animals. Blood glucose levels were measured 72 hours after injection using a glucometer (Accu-Chek® Instant S). Blood glucose levels above 250 mg/dL were considered diabetic (Furman, 2015). Diabetic animals were maintained for six weeks prior to creation of the wound model.

Wound model

The rats were anesthetized with IP ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg). The animals were placed in the dorsal decubitus position, the dorsal area shaved, and the shaving area treated with 70% alcohol. Three circular wounds, right and left, were created by removing the epidermis using a 6.0-mm biopsy punch (Acu-punch®, Acuderm) (Kaltalıoğlu et al., 2020).

Experimental groups

Rats were randomly divided into 4 groups. Each group consisted of 6 animals:

Group Control (C): Healthy animals were wounded only.

Group Diabetes (D): Diabetic animals were created with STZ, which were wounded six weeks later, but not treated.

Group Control NAC treatment (CNAC): Healthy animals were wounded and subsequently injected with IP NAC (60 mg/kg, prepared daily in 0.9% NaCl solution) for 7 days.

Group Diabetes + NAC treatment (DNAC): Diabetic animals were created with STZ, which were wounded six weeks later, and subsequently treated with IP NAC injection for 7 days.

Postoperative analgesia was provided by daily 0.04 mg/kg SC buprenorphine injection in wounded animals.

Morphological examination

The lesions were photographed using a Canon 400D dSLR camera on days 0 and 7 to assess wound contraction. For all shots, a

tripod was used to fix the camera–wound distance. The wound area was evaluated using the ImageJ software (National Institutes of Health, USA).

Seven days after the wound was created, the animals were sacrificed under anaesthesia and the wound tissues were immediately removed.

MDA measurement

As an indicator of lipid peroxidation, the level of malondialdehyde (MDA) in wound tissue was determined according to the spectrophotometric method described by Buege and Aust (1978).

GSH measurement

A modified Elman method was used to determine the reduced glutathione in tissue samples (Aykaç et al., 1985).

NOx measurement

The NOx levels in the samples were determined by the spectrophotometric method described by Miranda et al. (2001).

SOD activity measurement

SOD activity in the wound tissue was determined using the method described by Sun et al. (1988).

CAT activity measurement

The method developed by Aebi (1984) was used to determine CAT activity.

Determination of the collagen level in wound tissue

The total amount of collagen (types I–V) in the wound tissues was determined using a commercial kit (Sircol™ Soluble Collagen Assay).

Determination of Tumor Necrosis Factor-α (TNF-α), Interleukin 1- β (IL-1β), and Interleukin- 6 (IL-6) levels

TNF-α, IL-1β, and IL-6 levels in the supernatant of wound tissue homogenate were determined by commercial ELISA kits (Rat TNF-α Platinum ELISA, Affymetrix eBioscience; Rat IL-1β ELISA kit, eBioscience; Rat IL-6 ELISA kit, eBioscience) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA) for Microsoft Windows 10. All values are expressed as the mean ± SD; $p<0.05$ was considered significant. Kruskal-Wallis test was used to compare the wound area between the groups, followed by the Wilcoxon sign test for comparisons between wound areas on days 0 and 7. Kruskal Wallis test was used to compare biochemical parameters and collagen amounts between groups.

3. Results

MDA levels

The MDA levels are shown in Figure 1 The highest MDA level in wound tissue was found in diabetic animals. NAC administration significantly reduced the MDA level in wound tissue in Groups CNAC and DNAC as compared with their own controls ($p<0.05$).

GSH levels

As seen in Figure 2, the GSH level in wound tissue changed in parallel with enzymatic antioxidative activities. The wound tissue GSH level was highest in Group CNAC. The GSH level decreased in diabetic wounds; however, following NAC application, Group DNAC (3.21 ± 0.17 μmol/g tissue) showed a significant increase ($p<0.05$).

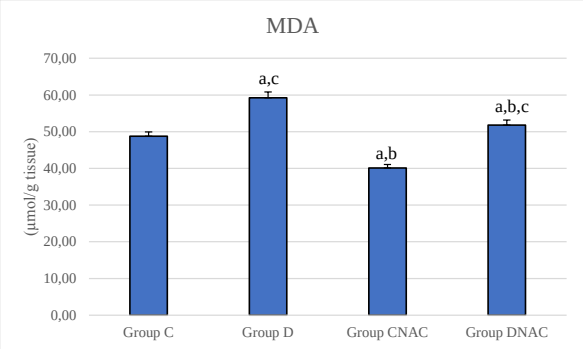

Figure 1. The effects of NAC treatment on the lipid peroxidation levels of wound tissue. [a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

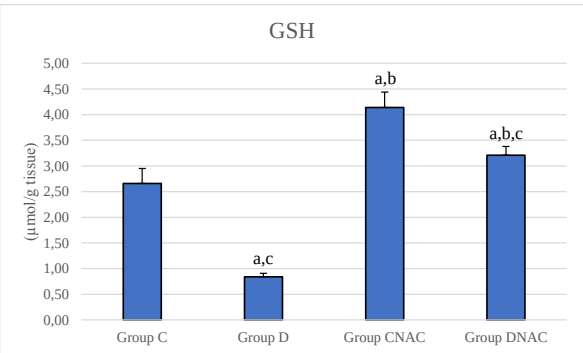

Figure 2. The effects of NAC treatment on the GSH levels of wound tissue.

[a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

Enzymatic antioxidant activities

The SOD and CAT activities are shown in Figure 3A and 3B respectively. The highest SOD and CAT enzyme activities in wound tissues were found in Group CNAC, which were significantly increased as compared with the control ($p<0.05$). In Group D, SOD and CAT enzyme activities in wound tissue were decreased remarkably due to diabetes. In Group DNAC wound tissue SOD and CAT enzyme activities were increased as compared with those in Group D ($p<0.05$).

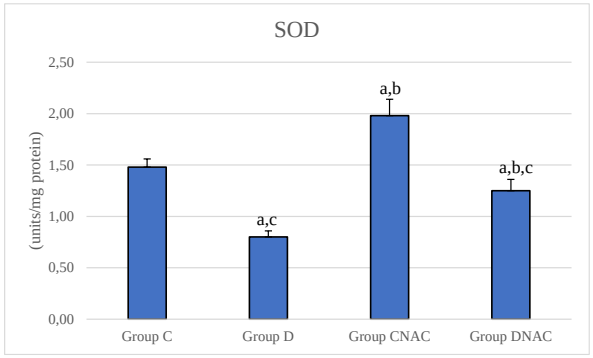

Figure 3A. The effects of NAC treatment on the SOD activity of wound tissue.

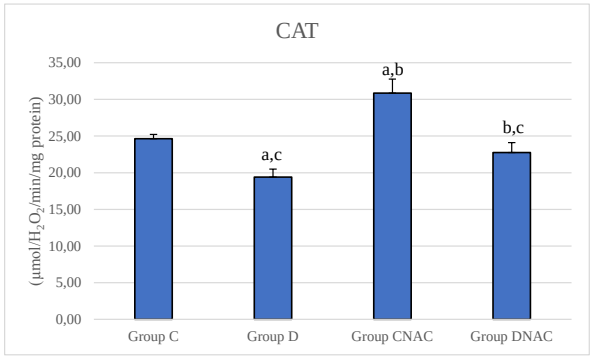

Figure 3B. The effects of NAC treatment on the CAT activity of wound tissue.

[a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

NOx levels

As shown in Figure 4, the highest NOx level in wound tissue was measured in Group C. The NOx level was significantly decreased in Groups D, CNAC, and DNAC as compared with that in Group C ($p<0.05$). In Group D, the NOx level was significantly decreased as compared with Group C ($p<0.05$) due to diabetes. NAC administration significantly increased the NOx level in wound tissue in Group DNAC as compared with Group D ($p<0.05$).

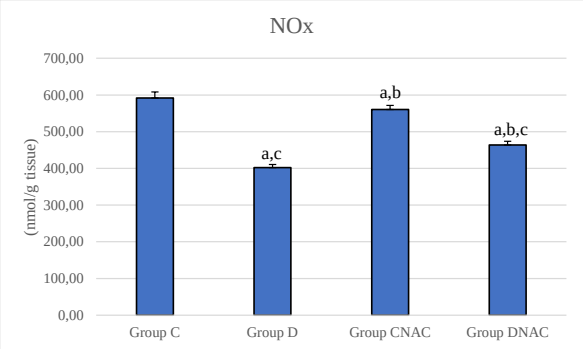

Figure 4. The effects of NAC treatment on the NOx levels of wound tissue.

[a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

Collagen quantity

As viewed in Figure 5, the quantity of wound tissue collagen was dramatically reduced in Group D as compared with Group C ($p<0.05$). NAC treatment significantly increased the amount of collagen in both Groups CNAC and DNAC as compared with their own controls ($p<0.05$).

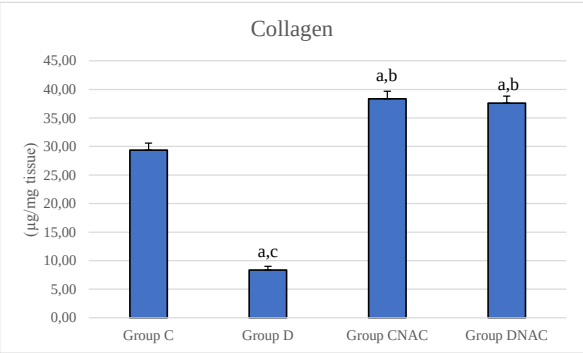

Figure 5. The effects of NAC treatment on the Collagen levels of wound tissue.

[a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

TNF-α, IL-1β, and IL-6 levels

The results presented in Figure 6A, 6B and 6C indicated that inflammatory markers (TNF-α, IL-1β, and IL-6) were significantly increased in Group D ($p<0.05$) as compared with Group C. However, in Group DNAC, the levels of inflammatory markers were significantly decreased ($p<0.05$).

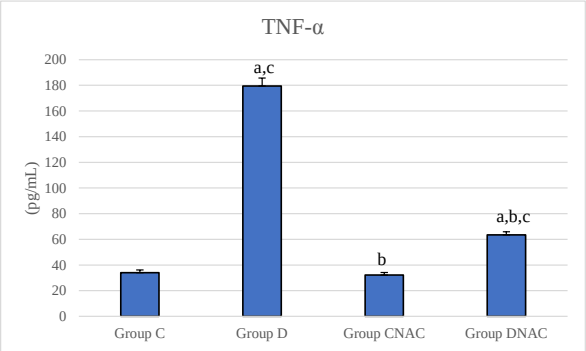

Figure 6A. The effects of NAC treatment on the TNF-α levels of wound tissue.

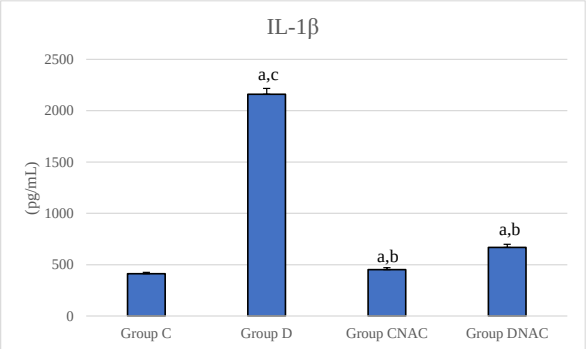

Figure 6B. The effects of NAC treatment on the IL-1β levels of wound tissue.

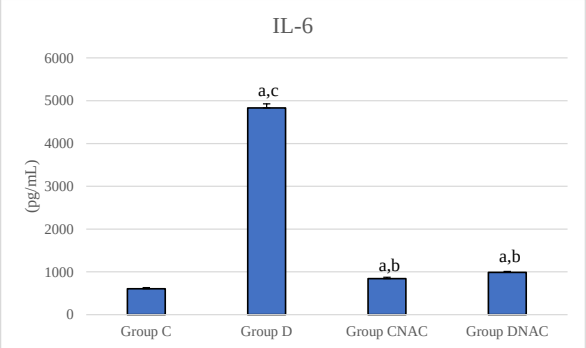

Figure 6C. The effects of NAC treatment on the IL-6 levels of wound tissue.

[a] $p<0.05$ compared to Group C, [b] $p<0.05$ compared to Group D, [c] $p<0.05$ compared to Group CNAC with the Kruskal Wallis Test

Fasting blood glucose (FBG) levels

Fasting blood glucose levels of all groups at the onset and the end of the experiment are presented in Figure 7. FBG levels of the Group D and Group DNAC were found to be significantly higher than the initial values (p<0.05).

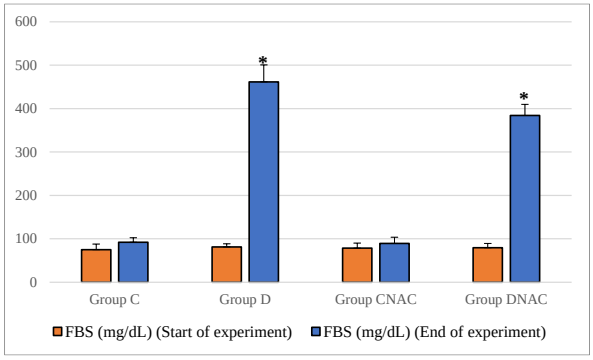

Figure 7 Effect of NAC on FBG levels. * p<0.05

Wound area and percentage wound contraction

According to the wound area and percentage wound contraction, significantly less improvement was observed in Group D as compared with Group C (p<0.05) (Table 1 and Figure 8). NAC treatment positively affected wound healing. The smallest wound area and highest wound contraction percentage were found in Group CNAC as compared with all other groups. In diabetic wounds, NAC treatment (Group DNAC) increased healing as indicated by a significantly smaller wound area and higher percentage wound contraction as compared with Group D (p<0.05).

Table 1. Comparison of wound areas at 0 and 7th days

Groups	Days	Wound area (mm^2)	Wound area p value [a]	Wound contraction (%)	Wound Contraction p value [b]
Group C	0 Day	28.31±0.27	0.01*	53.3%±11.2%	0.01*
	7th Day	13.20±0.50			
Group D	0 Day	29.08±0.16	0.01	27.2%±7.4%	0.01
	7th Day	20.63±0.31			
Group CNAC	0 Day	28.44±0.23	0.01*	58.9%±14.1%	0.01*
	7th Day	11.63±0.65			
Group DNAC	0 Day	26.11±0.42	0.01*	52.1%±10.7%	0.01*
	7th Day	13.56±0.57			

[a] Kruskall Wallis Test, [b] Wilcoxon Sign Test, *p<0.05 compared to Group D

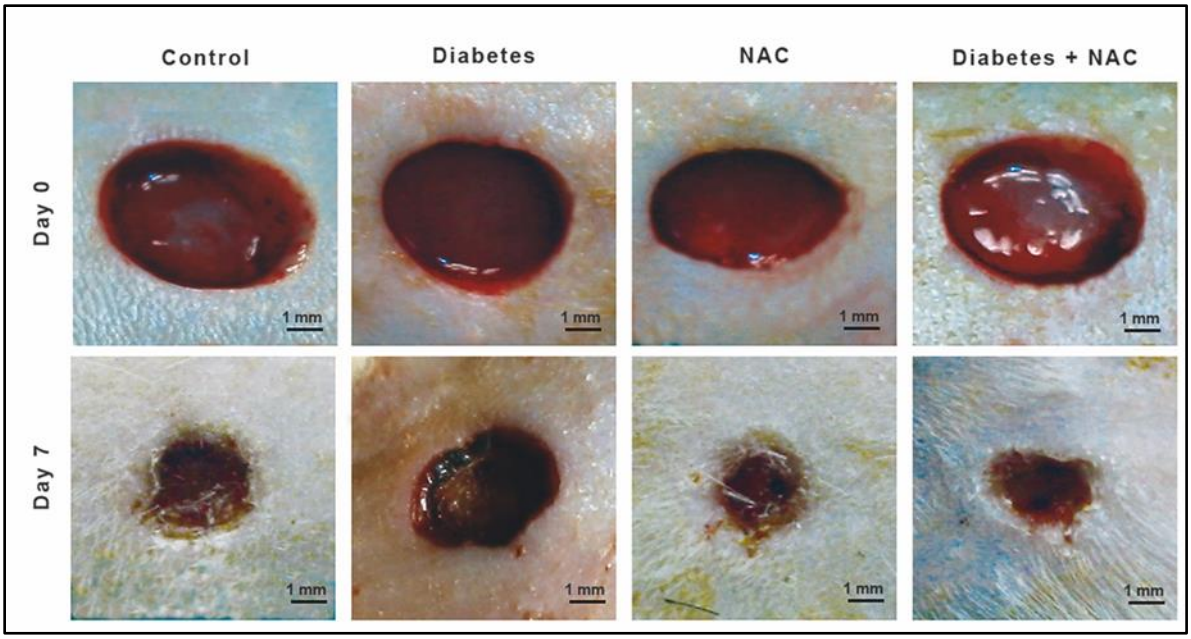

Figure 8. The effects of NAC treatment on the wound contractions of all groups at 0 and 7th days. Control as Group C; Diabetes as Group D; NAC as Group CNAC; Diabetes + NAC as Group DNAC.

4. Discussion

It is known that increased ROS production causes cellular damage and plays a role in the pathological development of diabetes (Luc et al., 2017); however, ROS produced at low concentrations creates a suitable environment in the inflammatory phase to initiate the wound healing process. The inflammatory phase of wound healing, the onset and cessation of which are tightly controlled by cellular mechanisms, is highly sensitive to the redox state of the wound (Kim et al., 2019). Overproduction or inadequate elimination of ROS causes oxidative damage by disrupting the redox state of the wound. In addition, the accumulation of AGEs also leads to increased oxidative stress (Luc et al., 2017). It has been suggested that increased oxidative stress is one of the underlying molecular mechanisms of impaired wound healing in diabetes (Cano-Sanchez et al., 2018). It has been reported that oxidative stress, which is known to be increased in diabetic wound healing, may lead to a stronger inflammatory response or a longer-lasting inflammatory phase (Luc et al., 2017; Cano-Sanchez et al., 2018; Kim et al., 2019). However, a reduction in, or impaired activity of, antioxidant molecules may also be the cause of impaired wound healing in diabetes (Ezhilarasu et al., 2020). NAC is an acetylated cysteine derivative that can help to protect cells against oxidative damage, and has been shown to modulate oxidative stress and inflammation in several ways (Janeczek et al., 2019). NAC easily passes through the cell membrane and becomes rapidly hydrolysed to L-cysteine, the precursor of reduced glutathione. It has been suggested that NAC contributes to an increase in the GSH pool, which is a major intracellular antioxidant. Therefore, NAC can normalize the impaired redox state of cells and affect redox-sensitive cell signalling and transcription pathways. The -SH group of NACs directly eliminates ROS. In addition, NAC has anti-inflammatory properties, manifested by the inhibition of pro-inflammatory cytokines such as IL-8, IL-6, and TNF-α (Aldini et al., 2018). NAC has

also been reported to have positive effects on wound healing. Demir et al. (2011) demonstrated that administration of IP NAC (300 mg/kg for 11 days) increases tissue hydroxyproline levels in anastomotic wounds after radiotherapy; increased serum GSH and SOD levels and decreased serum lipid peroxidation were also found. Yılmaz et al. (2015) showed that postoperative administration of IP NAC (300 mg/kg for 15 days) during nasal mucosal healing in rats accelerates healing by reducing inflammatory markers and goblet cell loss. In addition to its role in normal wound healing, several studies have shown that NAC application also has an accelerating effect on diabetic wound healing. Aktunc et al. (2010) reported that in alloxan-induced diabetic mice, IP NAC (150 mg/kg for 5 days) treatment reduces oxidative stress parameters and iNOS expression in wound tissue. An increase in vascular endothelial growth factor (VEGF) expression, wound breaking strength (WBS), and tissue GSH levels was also seen. Consequently, it has been suggested that NAC exerts a positive clinical effect by shortening the wound healing time. Özkaya et al. (2019) demonstrated that both topical (300 mg/kg for 14 days) and systemic (200 mg/kg for 14 days) treatment with NAC accelerates wound healing due to its positive effects on oxidative stress parameters in tissue and serum in diabetic rat models. Zayed et al. (2017) showed that NAC (150 mg/kg for 7 days) accelerates wound healing by increasing proliferation and reducing inflammation through perfusion and palmitoylation signalling of phospholipase Cβ and Gαq in endothelial cells under ischemic conditions in the STZ-induced diabetes model. On the other hand, Tsai et al. (2014) have reported that at the relatively lower doses than other studies various NAC concentrations (0.1, 0.5, and 1.0 mM) increased glutathione levels, cell viability, scratch-wound healing activities and migration abilities of CCD-966SK cells after 24 hours in a dose-dependent manner. In the present study, the effectiveness of low-dose and short-term NAC administration on diabetic wound healing, based on Tsai et al.'s research was evaluated. The results of the present study, in parallel with other findings in the literature, show that administration of a low dose of NAC (60 mg/kg) to diabetic animals for 7 days made a positive contribution to wound healing. The levels of SOD, catalase, and GSH in the wound tissue in the diabetic control group were significantly decreased as compared with those in other groups. The reduction in these antioxidants may be an indication that the endogenous antioxidant system cannot provide proper feedback due to the diabetic state. MDA levels may have increased through a poor antioxidative response in the diabetic group, contributing to the increment in oxidative stress. Moreover, weak wound contraction in the diabetic group may have been the result of increased oxidative stress. On the other hand, systemic NAC administration increased the levels of both enzymatic antioxidants and GSH and decreased lipid peroxidation as compared with the untreated groups. These results suggest that NAC administration can reduce lipid peroxidation in the wounds of diabetic rats by removing free radicals through the upregulation of components of the antioxidant system, thereby reducing tissue damage and accelerating healing. These positive contributions may have occurred through the direct effect of NAC or its metabolite, cysteine. It seems likely that systemic NAC administration may contribute to an increase in the GSH pool, normalizing the impaired redox state of cells and affecting redox-sensitive cell signalling and

transcription pathways. NO is an important transmitter synthesized from the amino acid L-arginine through nitric oxide synthase enzymes (eNOS, iNOS, and nNOS). NO plays a key role in the management of the three main parts of the wound healing process: vascular homeostasis, inflammation, and antimicrobial effect (Malone-Povolny et al., 2019). Abnormal NO production, which is a property of the diabetic state, is associated with delayed wound healing and the establishment of chronic wounds (Li and Lee, 2010). Aktunç et al. (2010) reported that NAC treatment (150 mg/kg for 5 days) suppresses iNOS activity within full-thickness incision wounds in alloxan-induced diabetic mice. On the contrary, the results of the present study show that NAC (60 mg/kg for 7 days) therapy increased the wound NOx level in the diabetic group. These differences may be due to the type of wound, the dose and duration of NAC administration, or the activities of different NOS isoforms. Previous findings demonstrate that diabetic wound fluid has a lower NO concentration than that of normal wound fluid due to downregulated eNOS (Witte et al., 2002). Macrophages, keratinocytes, and fibroblasts express a high level of iNOS at normal wound sites but this expression is reduced in diabetes. These cells express lower levels of iNOS and eNOS genes and cannot synthesize collagen or generate extracellular matrix (ECM). Changed fibroblast activity in diabetes results in decreased NO concentrations in the extracellular wound fluid, reduced amounts of collagen, and weak WBS (Kitano et al., 2017). In parallel with the change in NOx levels, the amount of collagen in wound tissue was reduced in diabetic wounds. As a result of NAC treatment, the amount of collagen was augmented in wounds. The NOx level in the NAC-treatment group remained lower than that in the control group, which may suggest that NAC exerts its effect through a mechanism other than changing fibroblast activity. An alternative mechanism may be the induction of epithelial cell proliferation. Zayed et al. (2017) showed that NAC (150 mg/kg for 7 days) accelerates wound healing by increasing perfusion and proliferation under ischemic conditions in mice with STZ-induced diabetes, suggesting that NAC increases the proliferation of endothelial cells through phospholipase Cβ signalling. In another study, it was reported that NAC inhibits inflammatory factors and facilitates epidermal proliferation via NF-κB (Samuni et al., 2013). On the other hand, overproduction of ROS, along with low levels of GSH in wound tissue, may have prevented collagen synthesis and accumulation through a different mechanism. Tissue collagen is affected by the presence of antioxidant molecules in wound tissue (Wu et al., 2011). It has been reported that following injury, tissue GSH levels are decreased, resulting in decreased collagen accumulation (Deveci et al., 2005). The results of the present study indicate that the amount of collagen may have increased as a result of increased antioxidative capacity. Various types of growth factors and pro-inflammatory cytokines play important roles in the regulation of the wound healing process. Pro-inflammatory cytokines, such as NF-κB, IL-1β, IL-6, and TNF-α, are essential during the initial response of the inflammatory phase of wound healing. Under physiological conditions, the presence of pro-inflammatory cytokines is important for the migration of neutrophils and the occurrence of respiratory burst at the wound site. Furthermore, these cytokines are powerful stimulators of metalloproteinase (MMP) synthesis, which break up and eliminate damaged extracellular matrix to support

wound healing in fibroblasts and inflammatory cells (Fard et al., 2015). However, as in diabetes, if the inflammatory phase is prolonged during the wound healing process, this causes the overproduction of pro-inflammatory cytokines, which are in high concentrations in the wound area, damaging the tissue and causing the wound to evolve into a chronic state (Li et al., 2020). Increased pro-inflammatory cytokine levels in diabetic wound healing have been reported in several studies (Ponrasu et al., 2012; Muhammad et al., 2016; Li et al., 2020). The anti-inflammatory effects of NAC have been shown under different pathological conditions in the kidney, brain, and liver (de Andrade et al., 2015; Mikolka et al., 2016; Schneider et al., 2017; Güntürk et al., 2019). The results of the present study show that IL-1β, IL-6, and TNF-α levels were increased in diabetic wounds without treatment. Following NAC treatment, a dramatic decrease in IL-1β, IL-6, and TNF-α levels were seen. Based on these results, it can be suggested that NAC administration may have had an anti-inflammatory effect, preventing the prolongation of the inflammatory phase despite the diabetic state. It is known that an increased inflammatory response is associated with oxidative stress. The direct and indirect (GSH synthesis) antioxidant efficiency of NAC, in addition to the antioxidant effects caused by disulphide bond breaking ability, may have prevented an abnormal inflammatory response by reducing oxidative stress. On the other hand, Moreno et al. (2014) reported that protein cysteinylation and oxidation of low-molecular weight thiols are defined as disulphide stress, which is also associated with inflammation. According to the ability of NAC to break thiolated proteins, this may have increased its anti-inflammatory properties by reducing disulphide stress, which is associated with inflammation. In addition to its antioxidant properties, several clinical and experimental studies have investigated the effects of NAC as a therapeutic agent against insulin resistance, type 2 diabetes, and related complications. Kaneto et al. (1999) reported that NAC has protective effects on pancreatic β cells in alloxan-induced diabetic mice. Moreover, Riberio et al. (2011) showed that NAC has a modulating effect on oxidative stress biomarkers in pancreatic cells. It has also been reported that NAC therapy reduces insulin resistance in insulin-resistant rats fed a high-carbohydrate diet (Ismael et al., 2008). The beneficial effect of NAC in the present study may also be related to its effects on glycaemic control in the diabetic rat model; however, this effect could not be verified here because the experimental design did not compare groups in terms of glycaemic control degree or insulin resistance. In the present study, the positive effects of NAC on wound healing in diabetic rats were mainly due to antioxidant activity.

The results of the present study indicate that a low dose of systemic NAC therapy had positive effects on wound healing in a diabetic rat model. This efficacy of NAC may be dependent on its antioxidative characteristics since a decrease in oxidative stress parameters was observed during the wound healing process. Due to the antioxidant effects of NAC, the elongated inflammatory phase that occurs during diabetes could be completed and wound healing continued its normal process.

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5. References

Adil M., Amin S.S., Mohtashim M., 2018. "N-acetylcysteine in dermatology", *Indian J. Dermatol. Venereol. Leprol.* 84, 652-659.

Aebi H., 1984. "Catalase in vitro", *Methods Enzymol.* 105, 121-126.

Aktunc E., Ozacmak V.H., Ozacmak H.S., Barut F., Buyukates M., Kandemir O., Demircan N., 2010. "N-acetyl cysteine promotes angiogenesis and clearance of free oxygen radicals, thus improving wound healing in an alloxan-induced diabetic mouse model of incisional wound", *Clin. Exp. Dermatol.* 35, 902-909.

Aldini G., Altomare A., Baron G., Vistoli G., Carini M., Borsani L., Sergio F., 2018. "N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why", *Free Radi.c Res.* 52, 751-762.

AlMatar M., Batool T., Makky E.A., 2016. "Therapeutic Potential of N-Acetylcysteine for Wound Healing, Acute Bronchiolitis, and Congenital Heart Defect", *Curr. Drug. Metab.* 17, 156-167.

Aykaç G., Uysal M., Yalçin A.S., Koçak-Toker N., Sivas A., Öz H., 1985. "The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats" *Toxicology*. 36, 71-76.

Boniakowski A.E., Kimball A.S., Jacobs B.N., Kunkel S.L., Gallagher K.A., 2017. "Macrophage-Mediated Inflammation in Normal and Diabetic Wound Healing", *J. Immunol.* 199, 17-24.

Brocke T. and Barr J., 2020. "The History of Wound Healing" *Surg. Clin. North. Am.*, 100, 787-806.

Buege J.A. and Aust S.D., 1978. "Microsomal lipid peroxidation". *Methods Enzymol*. 52, 302-310.

Cano-Sanchez M., Lancel S., Boulanger E., Neviere R., 2018. "Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review", *Antioxidants (Basel)*. 7, 98.

de Andrade K.Q., Moura F.A., dos Santos J.M., de Araújo O.R., de Farias Santos J.C., Goulart M.O., 2015. "Oxidative Stress and Inflammation in Hepatic Diseases: Therapeutic Possibilities of N-Acetylcysteine", *Int. J. Mol. Sci*. 16, 30269-30308.

Demir E.O., Cakmak G.K., Bakkal H., Turkcu U.O., Kandemir N., Demir A.S., Tascilar O., 2011. "N-acetyl-cysteine improves anastomotic wound healing after radiotherapy in rats", *J. Invest. Surg.* 24, 151-158.

den Dekker A., Davis F.M., Kunkel S.L., Gallagher K.A., 2019. "Targeting epigenetic mechanisms in diabetic wound healing", *Transl. Res*. 204, 39-50.

Deveci M., Gilmont R.R., Dunham W.R., Mudge B.P., Smith D.J., Marcelo C.L., 2005. "Glutathione enhances fibroblast collagen contraction and protects keratinocytes from

apoptosis in hyperglycaemic culture", *Br. J. Dermatol*. 152: 217-224.

Dunnill C., Patton T., Brennan J., Barrett J., Dryden M., Cooke J., Leaper D., Georgopoulos N.T., 2017. "Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process", *Int. Wound J.* 14, 89-96.

Ezhilarasu H., Vishalli D., Dheen S.T., Bay B.H., Srinivasan D.K., 2020. "Nanoparticle-Based Therapeutic Approach for Diabetic Wound Healing", *Nanomaterials (Basel)*. 10, 1234.

Fard M.T., Arulselvan P., Karthivashan G., Adam S.K., Fakurazi S., 2015. "Bioactive Extract from *Moringa oleifera* Inhibits the Pro-inflammatory Mediators in Lipopolysaccharide Stimulated Macrophages", *Pharmacogn. Mag*. 11, 556-563.

Fui L.W., Lok M.P.W., Govindasamy V., Yong T.K., Lek T.K., Das A.K., 2019. "Understanding the multifaceted mechanisms of diabetic wound healing and therapeutic application of stem cells conditioned medium in the healing process", *J. Tissue Eng. Regen. Med.* 13, 2218-2233.

Furman B.L., 2015. "Streptozotocin-Induced Diabetic Models in Mice and Rats" *Curr. Protoc. Pharmacol.* 70, 5.47.1-5.47.20.

Güntürk I., Yazici C., Köse S.K., Dağli F., Yücel B., Yay A.H., 2019. "The effect of N-acetylcysteine on inflammation and oxidative stress in cisplatin-induced nephrotoxicity: a rat model", *Turk J. Med. Sci*. 49, 1789-1799.

Ismael M.A., Talbot S., Carbonneau C.L., Beauséjour C..M, Couture R., 2008. "Blockade of sensory abnormalities and kinin B(1) receptor expression by N-acetyl-L-cysteine and ramipril in a rat model of insulin resistance", *Eur. J. Pharmacol*. 589, 66-72.

Janeczek M., Moy L., Riopelle A., Vetter O., Reserva J., Tung R., Swan J., 2019. "The Potential Uses of N-acetylcysteine in Dermatology: A Review", *J. Clin. Aesthet. Dermatol*. 12, 20-26.

Kaltalioglu, K., Balabanli, B., Coskun-Cevher, S. (2020). "Phenolic, Antioxidant, Antimicrobial, and In-vivo Wound Healing Properties of Potentilla erecta L. Root Extract in Diabetic Rats" *Iran. J. Pharm. Res.* 19, 264-274.

Kaneto H., Kajimoto Y., Miyagawa J., Matsuoka T., Fujitani Y., Umayahara Y., Hanafusa T., Matsuzawa Y., Yamasaki Y., Hori M., 1999. "Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity", *Diabetes.* 48, 2398-2406.

Kim J.H., Yang B., Tedesco A., Lebig E.G.D., Ruegger P.M., Xu K., Borneman J., Martins-Green M., 2019. "High Levels of Oxidative Stress and Skin Microbiome are Critical for Initiation and Development of Chronic Wounds in Diabetic Mice", *Sci. Rep*. 9, 19318.

Kitano T., Yamada H., Kida M., Okada Y., Saika S., Yoshida M., 2017. "Impaired Healing of a Cutaneous Wound in an Inducible Nitric Oxide Synthase-Knockout Mouse", *Dermatol. Res. Pract.* 2017, 2184040.

Lasram M.M., Dhouib I.B., Annabi A., El Fazaa S., Gharbi N., 2015. "A review on the

possible molecular mechanism of action of N-acetylcysteine against insulin resistance and type-2 diabetes development", *Clin. Biochem.* 48, 1200-1208.

Li J., Chou H., Li L., Li H., Cui Z., 2020. "Wound healing activity of neferine in experimental diabetic rats through the inhibition of inflammatory cytokines and nrf-2 pathway", *Artif. Cells Nanomed. Biotechnol*. 48, 96-106.

Li W., Li W., Leng Y., Xiong Y., Xue R., Chen R., Xia Z., 2020. "Mechanism of N-acetylcysteine in alleviating diabetic myocardial ischemia reperfusion injury by regulating PTEN/Akt pathway through promoting DJ-1", *Biosci. Rep.* 40, BSR20192118.

Li Y. and Lee P.I., 2010. "Controlled nitric oxide delivery platform based on S-nitrosothiol conjugated interpolymer complexes for diabetic wound healing", *Mol. Pharm.* 7, 254-266.

Luc K., Schramm-Luc A., Guzik T.J., Mikolajczyk T.P., 2017. "Oxidative stress and inflammatory markers in prediabetes and diabetes", *J. Physiol. Pharmacol*. 70, doi: 10.26402/jpp.2019.6.01.

Malone-Povolny M.J., Maloney S.E., Schoenfisch M.H., 2019. "Nitric Oxide Therapy for Diabetic Wound Healing", *Adv. Healthc. Mater.* 8, e1801210.

Mikolka P., Kopincova J., Mikusiakova L.T., Kosutova P., Calkovska A., Mokra D., 2016. "Antiinflammatory Effect of N-Acetylcysteine Combined with Exogenous Surfactant in Meconium-Induced Lung Injury", *Adv. Exp. Med. Biol*. 934, 63-75.

Miranda K.M., Espey M.G., Wink D.A., 2001. "A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite", *Nitric Oxide*. 5, 62-71.

Moreno M.L., Escobar J., Izquierdo-Álvarez A., Gil A., Pérez S, Pereda J., Zapico I., Vento M., Sabater L., Marina A., Martínez-Ruiz A., Sastre J., 2014. "Disulfide stress: a novel type of oxidative stress in acute pancreatitis", *Free Radic. Bio.l Med*. 70, 265-277.

Muhammad A.A., Arulselvan P., Cheah P.S., Abas F., Fakurazi S., 2016. "Evaluation of wound healing properties of bioactive aqueous fraction from *Moringa oleifera Lam* on experimentally induced diabetic animal model", *Drug Des. Devel. Ther.* 10, 1715-1730.

Ozkaya H., Omma T., Bag Y.M., Uzunoglu K., Isildak M., Duymus M.E., Kismet K., Senes M., Fidanci V., Celepli P., Hucumenoglu S., Aral Y., 2019. "Topical and Systemic Effects of N-acetyl Cysteine on Wound Healing in a Diabetic Rat Model", *Wounds*. 31, 91-96.

Ponrasu T. and Suguna L., 2012. "Efficacy of *Annona squamosa* on wound healing in streptozotocin-induced diabetic rats" *Int. Wound J*. 9, 613-623.

Ribeiro G., Roehrs M., Bairros A., Moro A., Charão M., Araújo F., Valentini J., Arbo M., Brucker N., Moresco R., Leal M., Morsch V., Garcia S.C., 2011. "N-acetylcysteine on oxidative damage in diabetic rats", *Drug and Chemical Toxicology*. 34, 467-474.

Samuni Y., Goldstein S., Dean O.M., Berk M., 2013. "The chemistry and biological activities of N-acetylcysteine", *Biochim. Biophys. Acta.*1830, 4117-4129.

Schneider R. Jr., Bandiera S., Souza D.G., Bellaver B., Caletti G., Quincozes-Santos A., Elisabetsky E., Gomez R., 2017. "N-acetylcysteine Prevents Alcohol Related Neuroinflammation in Rats", *Neurochem. Res.* 42, 2135-2141.

Shen F.C., Weng S.W., Tsao C.F., Lin H.Y., Chang C.S., Lin C.Y., Lian W.S., Chuang J.H., Lin T.K., Liou C.W., Wang P.W., 2018. "Early intervention of N-acetylcysteine better improves insulin resistance in diet-induced obesity mice", *Free Radic. Res.* 52, 1296-1310.

Sun Y., Oberley L.W., Li Y., 1988. "A simple method for clinical assay of superoxide dismutase", *Clin. Chem.* 34, 497-500.

Thi P.L., Lee Y., Tran D.L., Thi T.T.H., Kang J.I., Park K.M., Park K.D., 2020. "In situ forming and reactive oxygen species-scavenging gelatin hydrogels for enhancing wound healing efficacy", *Acta Biomater.*103, 142-152.

Tort S., Demiröz F.T., Coşkun Cevher Ş., Sarıbaş S., Özoğul C., Acartürk F., 2020. "The effect of a new wound dressing on wound healing: Biochemical and histopathological evaluation", *Burns*. 46, 143-155.

Tsai M.L., Huang H.P., Hsu J.D., Lai Y.R., Hsiao Y.P., Lu F.J., Chang H.R., 2014. "Topical N-acetylcysteine accelerates wound healing in vitro and in vivo via the PKC/Stat3 pathway", *Int. J. Mol. Sci.* 15, 7563-78.

Wang T., Mao X., Li H., Qiao S., Xu A., Wang J., Lei S.., Liu Z, Ng K.F., Wong G.T., Vanhoutte P.M., Irwin M.G., Xia Z., 2013. "N-Acetylcysteine and allopurinol up-regulated the Jak/STAT3 and PI3K/Akt pathways via adiponectin and attenuated myocardial postischemic injury in diabetes", *Free Radic. Biol. Med*. 63, 291-303.

Westby M., Norman G., Vedhara K., Game F., Cullum N., 2020. "Psychosocial and behavioural prognostic factors for diabetic foot ulcer development and healing: a systematic review", *Diabet. Med*. 37, 1244–1255.

Witte M.B., Thornton F.J., Tantry U., Barbul A., 2002. "L-Arginine supplementation enhances diabetic wound healing: involvement of the nitric oxide synthase and arginase pathways", *Metabolism*. 51, 1269-1273.

Wu G., Bazer F.W., Burghardt R.C., Johnson G.A., Kim S.W., Knabe D.A., Li P., Li X., McKnight J.R., Satterfield M.C., Spencer T.E., 2011. "Proline and hydroxyproline metabolism: implications for animal and human nutrition", *Amino Acids*. 40, 1053-1063.

Yilmaz B., Türkçü G., Şengül E., Gül A., Özkurt F.E., Akdağ M., 2015. "Efficacy of N-Acetylcysteine on Wound Healing of Nasal Mucosa" *J. Craniofac. Surg*. 26, e422-426

Zayed M.A., Wei X., Park K.M., Belaygorod L., Naim U., Harvey J., Yin L., Blumer K., Semenkovich C.F., 2017. "N-Acetylcysteine accelerates amputation stump healing in the setting of diabetes" *FASEB J.* 31, 2686-2695.