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Research Article

Reducing oxidative stress and enhancing antioxidant defenses for faster healing in diabetic wounds: The role of topical metformin

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Abstract

Objectives: Hyperglycemia, one of the most important metabolic indicators of diabetes, causes increased oxidative stress and inflammation both systemically and locally at the tissue level, particularly in chronic wound sites where healing is impaired. Increased oxidative stress products are controlled by the body's antioxidant capacity. Oxidative damage develops as a result of excessive production or improper quenching of reactive oxygen species (ROS) and is an important cause of non-healing chronic wounds. We aimed to accelerate wound healing by increasing the antioxidant capacity of oxidative damage caused by diabetes by applying metformin, which is routinely used orally, topically on wounds.

Methods: For this purpose, we applied metformin on diabetic and non-diabetic wounds for 14 days and measured oxidative stress markers malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS) levels, and antioxidant levels of glutathione (GSH) and catalase (CAT) in wound samples obtained by biopsy using ELISA technique.

Results: The markers of oxidative stress increased in untreated diabetic rats because of hyperglycemia, the most important clinical marker of diabetes, compared to non-diabetic rats. In contrast, our results showed that metformin administration decreased oxidative stress markers and increased antioxidant levels compared to controls.

Conclusion: As a result, it has been revealed that topically applied metformin can minimize oxidative damage caused by hyperglycemia by increasing antioxidant capacity, especially in diabetic wounds, and thus wounds heal faster by controlling oxidative stress in wound healing.

Keywords: Antioxidant, diabetes mellitus, metformin, oxidative stress, wound healing

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Diabetes is a metabolic disorder characterized by hyperglycemia due to insulin resistance, insufficient insulin secretion, or both [1]. It has been shown that hyperglycemia acts through pathways that include the polyol/aldose reduction pathway, the advanced glycation end product (AGE) pathway, the reactive oxygen species (ROS) pathway, and the protein kinase-C (PKC) pathway [2]. These pathways produce oxidant and inflammatory mediators that cause damage both locally in tissues and systemically. Inflammatory and oxidant pathways contribute to the pathogenesis of diabetic complications. Hyperglycemia is known to cause intracellular oxidative stress, and ROS that result from oxidative stress cause more damage to cells and delay healing in chronic or impaired acute wounds [3–5].

During wound healing, inflammatory cells, including macrophages, neutrophils, endothelial cells and fibroblasts, produce active oxygen and free radicals [6]. While adequate amounts of free radicals promote wound healing, too much

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active oxygen suppresses the migration and proliferation of repairing cells, inhibits extracellular matrix synthesis, and ultimately delays wound healing [7, 8]. Experimental and clinical studies have shown that delay in diabetic wounds increases cellular damage by excessive ROS production via the glucose autooxidation pathway, and this delayed wound healing is associated with oxidative stress [9–14].

In recent years, a variety of agents have been introduced to reduce the oxidant level and accelerate the wound healing process by increasing the antioxidant level [15–24]. Metformin, a biguanide derivative has been used for over 60 years in the treatment of the early stages of type 2 diabetes due to its ability to lower plasma glucose levels. Studies have been conducted to show its effectiveness against other diseases, including cancer (breast cancer, endometrial cancer, bone cancer, colorectal cancer and melanoma), obesity, liver diseases, cardiovascular diseases kidney diseases and even ageing, but the mechanisms underlying these different benefits remain unclear [25]. *In vitro* and *in vivo* studies have shown that metformin has a protective effect against oxidative damage caused by hyperglycemia and inhibits the expression of pro-inflammatory cytokines [26–28]. However, studies have yet to identify the effect of metformin on oxidative stress in wound healing.

It has been reported that a decrease in antioxidant levels and an increase in oxidant levels are important causes of delayed wound healing in diabetes [29, 30]. In this study, we investigated how to reverse the oxidative stress effects of hyperglycemia, one of the main complications of diabetes, on wounds on the 3rd, 7th and 14th days, which are the critical days in the wound healing process, by using topical metformin with its possible antioxidative effects. These days represent key stages in the wound healing process: inflammation (day 3), proliferation (day 7), and remodeling (day 14). During these phases, oxidative stress levels and antioxidant defenses exhibit dynamic changes. Day 3 is marked by high oxidative stress levels due to inflammatory cell activity, day 7 shows increasing antioxidant defenses supporting tissue regeneration, and by day 14, oxidative stress declines significantly while antioxidant systems peak to facilitate wound remodeling and closure [5, 6]. Current literature suggests that metformin may regulate inflammatory processes by suppressing important markers of oxidative stress, but the specific mechanisms in wound healing are still not fully elucidated. Our study shows that metformin strengthens antioxidant defense mechanisms by reducing oxidative stress load and thus accelerates wound healing. Thus, by applying metformin, which is known for its oral use, locally on the wound, we presented the potential to reduce the level of oxidative stress biomolecules and accelerate the wound healing process by increasing the level of antioxidative biomolecules. These findings emphasize that metformin may play a critical role not only in glucose regulation but also in cellular stress responses and offer a new treatment approach for wound healing. Topical use of metformin stands out as a therapeutic alternative for the wound healing process, especially with the advantage of avoiding systemic side effects.

Materials and Methods

Study design

An application was made to the Animal Experiments Local Ethics Committee of Bezmialem Vakif University to investigate the oxidative mechanism biomarker levels in tissues from experimental animals obtained in another research project. Approval was granted on 06.07.2022 according to committee decision E.69264. The study was designed in accordance with the Helsinki Declaration.

Tissue samples were taken from a previous study conducted by our group [31]. In the study, CG defines the control group, CT defines the healthy treatment group administered metformin, DCG defines the diabetic control group, and DTG defines the diabetic treatment group administered metformin. Each experimental group consisted of 6 rats. Physiological saline was applied to the control wounds and 3mM metformin was applied to the treatment groups by being absorbed into surgical sponges for 14 days. The absorption of metformin was standardized separately by pre-soaking the sponges in a metformin solution of defined concentration and volume, ensuring consistent dosing across all treatment groups. Levels of oxidative and antioxidative markers were investigated in wound tissues taken on days 0, 3, 7, and 14.

Tissue sampling and homogenization

Tissue samples from rats (n=24) were weighed, and then placed in polypropylene tubes; 1 mL of phosphate buffer (PBS, pH 7.4) was added to the samples of equal weight. Homogenization was carried out via homogenizer at 30 m/s for 5 minutes (FastPrep-24 homogenizer, MP Biomedicals, New Zealand). The homogenates were centrifuged at 10,000 x rpm for 15 minutes. Total protein quantitation was measured using the Bradford method [32]. All homogenates were stored at -80°C.

Oxidative stress marker assays

Total Antoxidant Status (TAS) and Total Oxidant Status (TOS) measurements

TAS and TOS levels were measured by spectrophotometry using commercial kits (Rel Assay, Türkiye). Antioxidants in the sample converted the dark blue-green ABTS radical to its colourless reduced form. The 660 nm absorbance change (Perkin Elmer, 1420 Victor 3 instrument, USA) was related to the total antioxidant level of the sample. Total antioxidant activity was expressed in mmol Trolox eq/L of samples. Oxidants present in the sample oxidized the ferrous ion chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion formed a colour complex with chromogen in an acidic medium. The colour intensity was related to the total amount of oxidant molecules present in the sample. Total oxidant activity was expressed in terms of μm H_2O_2 eq/L.

Determination of oxidative stress index (OSI)

The TOS to TAS ratio was used to calculate the oxidative stress index (OSI). The resulting TAS unit was converted to

mmol/l, and the OSI value was calculated according to the following formula [33]:

OSI (arbitrary unit) = TOS (μmol H_2O_2 eq/L) / TAS (mmol Trolox eq/L)

Catalase (CAT), Glutathione (GSH), and Malondialdehyde (MDA) measurements

Homogenates were thawed and Rat CAT ELISA kit (Mybiosource inc., USA, Cat Num: MBS726781), Rat GSH ELISA kit (Mybiosource inc., USA, Cat Num: MBS1600118) and Rat MDA ELISA kit (Mybiosource inc., USA, Cat Num: MBS738685 were used to quantitatively measure tissue sample levels. Briefly; tissue homogenates and standards were added to wells precoated with Anti-Rat monoclonal antibody before incubation; then biotin was added to all wells with Streptavidin-HRP to form the immune complex. Subsequent incubation and washing removed the uncombined enzyme. Chromogen Solutions A and B were added for the colour of the liquid changes into the blue in plates. At the effect of acid, the colour finally became yellow. A standard automated plate reader at 450 nm (Perkin Elmer, 1420 Victor3) was used to read optical density. The detection range of kits is between 0–50 ng/mL for CAT; 20–1600 mg/mL for glutathione and 0–1000 ng/mL for MDA. Samples were prepared at 4°C. Additionally, antioxidant and oxidant measurements were taken from all tissues simultaneously and without waiting, immediately after the homogenization of the tissues.

Statistical analysis

Statistical analysis was performed by using the software GraphPad Prism 8 version 8.4.3. The experimental data were expressed as mean ± standard deviation (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for pairwise comparisons. $p<0.05$ was considered statistically significant.

Results

TAS and TOS levels

TAS levels were measured on days 3, 7 and 14 of the wound healing process (Table 1). On day 3, no significant difference was found between the treatment groups of the control and diabetic groups. When the 7th-day TAS results were evaluated, the DTG group did show a significant increase compared to its control (DCG) (p=0.0011). Results obtained on the 14th day of wound healing were similar to results obtained on the 7th day. The DTG group showed a significant increase (p=0.0001) compared to its control, while the CT group showed no significant difference compared to its control.

TOS levels also showed significant changes over the healing period (Table 1). On day 3, the CT group and DTG group significantly decreased compared to their respective controls (p<0.0001, p<0.0001, respectively). Similar to the results of the 3rd day, the CT and DTG groups showed a significant decrease compared to their controls on the 7th day (p=0.001, p=0.0275, respectively). On the 14th day, unlike the other treatment days, only the diabetic treatment group showed significant improvement on day 14, highlighting the prolonged effect of metformin in diabetic wounds (p=0.0169).

Oxidative stress index (OSI)

The oxidative stress index (OSI) on the 3rd, 7th and 14th days of wound healing was recorded for the groups (Table 1, Fig. 1). On the 3rd and 7th days, the OSI level in the CT and DTG groups showed significance compared to the controls (p=0.0009, p=0.0007, p=0,0456, p=0.0002 respectively). While the CT group did not show a significant difference compared to the control on the 14th day, the DTG group continued its significant decrease compared to the control on the last day of treatment (p<0.0001).

MDA levels

MDA levels were recorded on the 3rd, 7th and 14th days of wound healing (Table 1, Fig. 1). When the CT group was compared to its control and the diabetic treatment group was compared to its control, both were found to be significantly reduced on the 3rd and 7th days (p<0.0001, p<0.0001, p=0.001, p=0.0273, respectively). A significant decrease was found comparing the diabetic treatment group on the 14th day to its control (p=0.0168). On day 14, the CT group did not show significant differences compared to its control, indicating limited effects of metformin in non-diabetic wounds.

Glutathione levels

Glutathione values (Table 1, Fig. 1) in the CT group were not significantly different compared to their controls in each other every three days. The metformin-treated diabetic group increased significantly on days 3, 7, and 14 compared to its control (p=0.0054, p<0.0001, p<0.0001, respectively).

CAT levels

The catalase values (Table 1, Fig. 1) in the health treatment group showed no significant difference compared to their controls in each other every 3 days. The diabetic group treated with metformin increased significantly on days 3, 7, and 14 compared to its control (p=0.0054, p<0.0001, p<0.0001, respectively).

Discussion

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the compensatory mechanisms of the endogenous antioxidant system. Antioxidant enzymes serve as the primary defense against the detrimental effects of free radicals. Key enzymes in this process include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase, which are abundant in the skin and play a crucial role in counteracting the harmful effects of excess ROS during the wound healing process [34]. In diabetic individuals, this antioxidant activity diminishes further as oxidative stress escalates, thereby increasing susceptibility to the damaging effects of free radicals [35]. In our study, catalase (CAT) for enzymatic antioxidant defense,

Table 1. TAS, TOS, calculated OSI, MDA, GSH and CAT levels of biopsy samples taken on days 0, 3, 7 and 14

Parameters	Days	Groups				
		CG	CT	DCG	DTG	p
Total oxidant status (TOS)	Day 0	6.953±0.8431		9.093±0.8624***		0.0002
	Day 3	9.38±0.4812	7.343±0.5698†	10.28±0.467§	8.359±0.63†,¶	<0.0001
	Day 7	9.655±0.6158	8.327±0.2775†	9.958±0.4901	9.058±0.5704†	<0.0001
	Day 14	8.852±0.8525	7.978±0.05675	9.522±0.467	8.528±0.2562†	0.0169
Total antioxidant status (TAS)	Day 0	1.101±0.04483		0.845±0.04806****		<0.0001
	Day 3	0.9517±0.07468	1.03±0.07127	0.77±0.09165§	0.7886±0.085¶	<0.0001
	Day 7	0.8433±0.06088	0.9233±0.8618	0.6917±0.0801§	0.9067±0.098†	0.0003
	Day 14	0.8833±0.07339	0.946±0.05727	0.6417±0.088§	0.8967±0.092†	<0.0001
Oxidative stress index (OSI)	Day 0	6.34±0.8751		10.75±0.6992****		<0.0001
	Day 3	9.917±1.033	7.162±0.853†	13.52±1.726§	10.7±1.578†,¶	<0.0001
	Day 7	11.57±1.483	9.09±0.8337†	14.63±2.244§	10.08±1.082†	<0.0001
	Day 14	10.03±0.9919	8.446±0.4998	15.1±2.37§	9.614±1.143†	<0.0001
Malondialdehyde (MDA)	Day 0	387.2±46.95		506.2±47.99***		0.0002
	Day 3	522.1±26.82	408.7±31.64†	572.4±25.88§	465.3±35.24†,¶	<0.0001
	Day 7	537.6±34.21	463.5±15.38†	554.4±27.32	504.3±31.81†	<0.0001
	Day 14	492.6±47.41	444.1±3.248	530.1±26.04	474.8±14.2†	0.0008
Glutathione (GSH)	Day 0	1205±209.7		755.5±244.6**		0.0016
	Day 3	880.7±207.9	985.1±190.4	474.5±70.45§	783.1±67.65†	<0.0001
	Day 7	764.2±107.9	771.3±106.4	318.2±91.25§	707.5±107.6†	<0.0001
	Day 14	751.6±39.36	828.4±41.11	442.2±46.63§	827.1±71.9†	<0.0001
Catalase (CAT)	Day 0	45.19±8.499		26.98±9.912**		0.0016
	Day 3	32.06±8.428	36.29±7.713	15.59±2.856§	28.1±2.741†	<0.0001
	Day 7	27.34±4.373	27.62±4.315	9.26±3.699§	25.04±4.362†	<0.0001
	Day 14	26.83±1.595	29.94±1.666	14.02±2.236§	29.48±3.25†	<0.0001

Significant levels of non-diabetic and diabetic groups treated with metformin compared to their controls on days 3, 7, and 14 are shown with the † symbol. In addition, the results of the non-diabetic and diabetic control groups without treatment were evaluated with the symbol § among themselves on the 3rd, 7th, and 14th days, and the results of the non-diabetic and diabetic treatment groups treated with metformin were evaluated with the symbol ¶ among themselves on the 3rd, 7th, and 14th days. The comparison of the diabetic groups to the non-diabetic group on day 0, which is the day the wound first opened, is marked with the * symbol (*p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001). CG: Untreated non-diabetic group; CT: Non-diabetic group treated with metformin; DCG: Untreated diabetic group; DTG: Diabetic group treated with metformin.

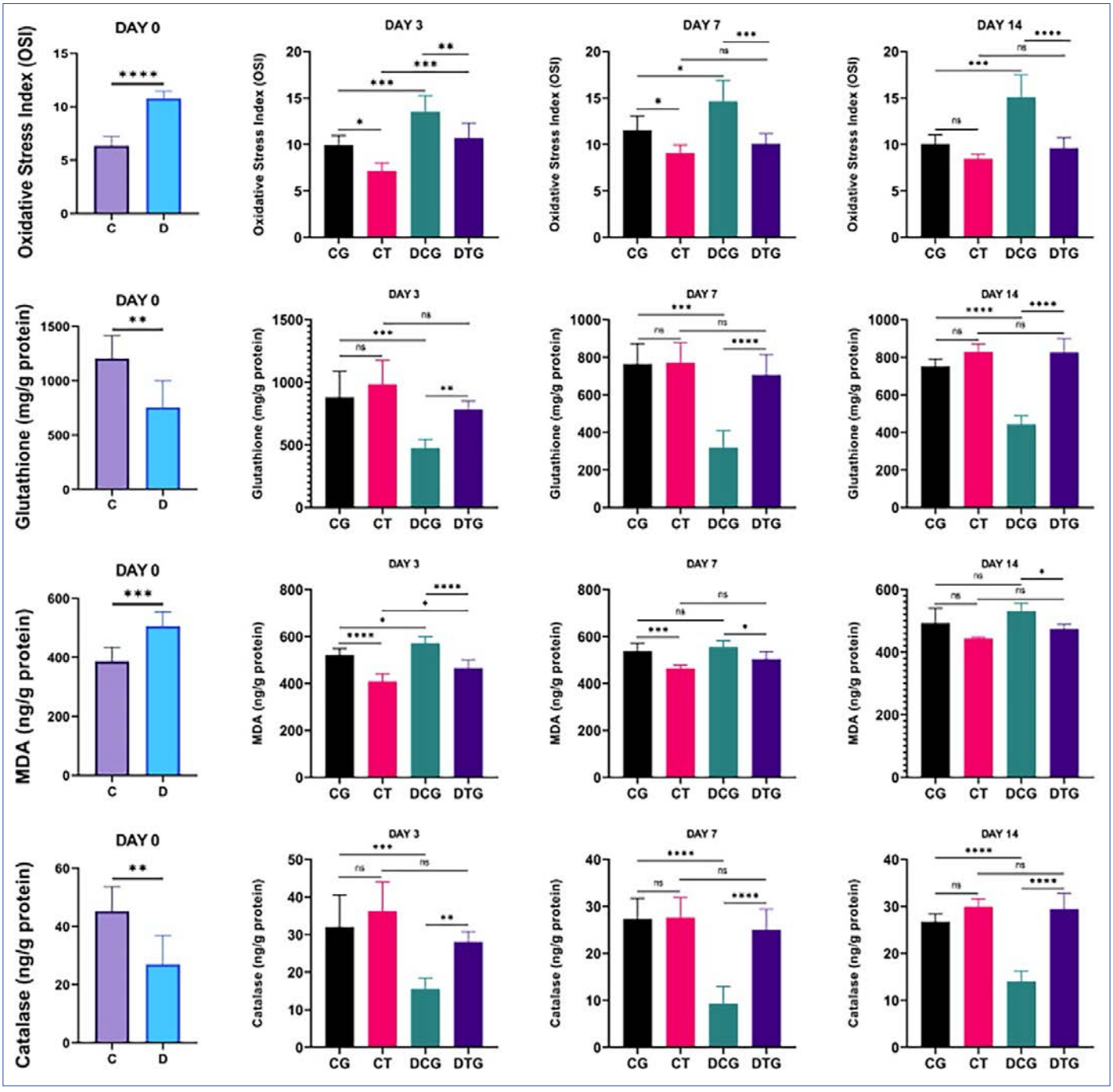

Figure 1. Comparative display of calculated OSI, MDA, GSH and CAT levels of biopsy samples taken on days 0, 3, 7 and 14.

ns: No significant; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001. CG: Untreated non-diabetic group; CT: Non-diabetic group treated with metformin; DCG: Untreated diabetic group; DTG: Diabetic group treated with metformin; OSI: Oxidative stress index; MDA: Malondialdehyde; GSH: Glutathione; CAT: Catalase.

glutathione (GSH) for non-enzymatic antioxidant capacity, and malondialdehyde (MDA) for lipid peroxidation were selected as they represent the key mechanisms in the oxidative stress pathway. CAT and GSH are primary antioxidants that neutralize reactive oxygen species (ROS) and protect cellular structures, while MDA is a well-known marker of lipid peroxidation indicating oxidative damage to cell membranes [24]. Collectively, these markers provide a comprehensive view of the oxidative-antioxidative balance in wound tissues.

Metformin, an established medication for type 2 diabetes, has garnered attention for its antioxidant properties, particularly through its activation of the AMP-activated protein kinase (AMPK) pathway. This activation enhances the expression of various antioxidant enzymes, thereby improving the body's ability to combat oxidative stress. The antioxidant effects of metformin are particularly significant in tissues where oxidative stress is prevalent, as it reduces reactive oxygen species (ROS) levels and improves overall antioxidant capacity [36,

37]. In both *in vitro* and *in vivo* studies, metformin has demonstrated a protective effect against oxidative damage induced by hyperglycemia and has been shown to inhibit the expression of pro-inflammatory cytokines [26–28]. While metformin is commonly administered orally, recent studies highlight the potential benefits of topical applications. Topical metformin has been shown to provide more effective localized healing that may exceed that achieved through oral administration, particularly in conditions such as diabetic wounds [38–40]. By delivering metformin directly to the affected tissue, this approach can effectively mitigate oxidative damage, enhance healing processes, and reduce systemic side effects associated with oral dosing. The localized application also might allow for higher concentrations of metformin at the site of injury, further promoting antioxidant activity and potentially accelerating the wound healing process. In summary, the antioxidant properties of metformin, combined with its effective topical application, present a promising avenue for enhancing therapeutic outcomes in oxidative stress-related conditions, including diabetic wounds. This approach not only utilizes metformin's inherent benefits but also maximizes its efficacy by targeting the specific areas of need directly. Our findings indicate that the topical application of metformin effectively reduces oxidative stress in diabetic wounds while concurrently elevating antioxidant levels.

Antioxidants are recognized for their protective effects against free radicals. Total antioxidant status (TAS) analysis serves as an important measure of tissue antioxidant capacity. The synergistic effects of various antioxidants provide enhanced defense against reactive oxygen and nitrogen species compared to individual compounds. Additionally, the oxidative stress index (OSI)—the ratio of total oxidant status (TOS) to TAS—reflects the oxidative stress level and redox balance within tissues [41, 42]. In wound model studies, such as those using Aloe vera and Hypericum perforatum (HPO), significant increases in TAS and non-significant changes in TOS have been observed in diabetic treatment groups, alongside reduced OSI levels compared to diabetic controls [43]. Our study similarly shows significant decreases in TOS in both non-diabetic and diabetic scar tissues on the 3rd and 7th days post-treatment, with comparable trends on the 14th day specifically in the diabetic group. The relative decrease in TOS was more pronounced in the diabetic treatment group, reflecting their higher baseline oxidative stress levels. This indicates that the treatment was more effective in the diabetic cohort, likely due to the elevated oxidative stress associated with diabetes. Moreover, TAS results revealed notable increases in antioxidant levels in diabetic wounds treated with metformin on the 7th and 14th days compared to controls, while no significant differences were observed in non-diabetic wounds. Interestingly, total antioxidant levels were higher in the diabetic group receiving metformin during later stages of healing. OSI values, indicating oxidative stress, significantly decreased in both diabetic and non-diabetic treatment groups on the 3rd and 7th days, with significant differences persisting only in the diabetic treatment group on the 14th day. These findings emphasize the efficacy of topical metformin application in reducing oxidative stress specifically in diabetic wounds compared to non-diabetic wounds, highlighting its therapeutic potential in enhancing wound healing. The increased efficacy in diabetic wounds may be attributed to elevated oxidative stress levels in diabetes, which make the antioxidant effects of metformin more impactful.

High levels of reactive oxygen species (ROS) lead to lipid oxidation, resulting in malondialdehyde (MDA) accumulation, which serves as a well-established biomarker for oxidative stress and cell membrane damage [44, 45]. Research by Yang et al. [46] indicates that increased oxidative stress delays wound healing, underscoring the importance of limiting ROS formation in diabetic patients to facilitate repair processes. Our findings corroborate these observations, demonstrating that elevated oxidative stress adversely affects the antioxidant/oxidant ratio, thereby hindering wound healing. Lima et al. [47] reported that Galactomannan GM-DR, known for its anti-inflammatory properties, reduced MDA levels by 44% on the 2nd day compared to controls administered saline, with no changes observed on the 5th and 14th days [47]. Additionally, studies utilizing diabetic rat wound models have shown that topical applications of bilirubin and deferoxamine significantly lowered MDA levels from day 7 to day 19, while topical quercetin showed similar effects on days 3 and 14 [48, 49]. Consistent with our findings, Assar et al. [50] observed a notable decrease in MDA levels in groups treated with licorice extract compared to untreated controls [50]. In our study, we compared each diabetic treatment group with their respective control groups and observed significant reductions in MDA levels, particularly on the 3rd and 7th days. The decrease in MDA levels across all treatment days suggests that the treatment was effective, especially on the 3rd day, likely due to the inflammatory response integral to the wound healing process. This highlights the potential of targeting oxidative stress to enhance healing outcomes in diabetic wounds.

Catalase, an enzyme prevalent in most aerobic cells, is essential for mitigating oxidative stress by catalyzing the rapid decomposition of hydrogen peroxide (H_2O_2) through its peroxidative and catalytic activities [51]. In both acute and chronic wounds, the activity of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase is often diminished due to elevated oxidative stress, which can overwhelm these antioxidants when released in significant quantities. Furthermore, high oxidative stress contributes to the depletion of non-enzymatic antioxidants, including vitamins E and C, as well as glutathione. This effect is more pronounced in chronic wounds compared to acute ones, indicating that antioxidant supplementation may help prevent cellular oxidative damage and enhance healing outcomes [52]. In a study involving diabetic rats, Afzali et al. [53] reported decreased levels of glutathione (GSH) alongside unaffected catalase activity and total antioxidant status (TAS) on the 7th day following treatment with acidic nitrite, which contradicted our findings. They also noted that these

parameters showed no significant changes on the 14th, 21st, and 28th days post-injury. Conversely, consistent with our results, other studies indicated that topical quercetin significantly increased CAT levels in diabetic wounds only on day 14 compared to controls, while bilirubin-deferoxamine treatment elevated CAT levels on days 3, 7, 14, and 19 compared to controls [48, 49]. Our study demonstrated that, although metformin treatment increased CAT levels in non-diabetic groups, the difference was not statistically significant. However, in the diabetic treatment group, there was a significant and consistent linear increase in CAT levels compared to controls across all biopsy days. This pronounced increase in the diabetic group suggests a substantial reduction in CAT levels due to diabetes, with metformin effectively reversing this decline and thereby widening the gap in CAT levels compared to the non-diabetic group. This reinforces the potential of metformin as a therapeutic agent to enhance antioxidant defenses in diabetic wound healing.

The intracellular redox buffer glutathione (GSH), a thiol antioxidant, plays a crucial role as a direct free radical scavenger, a co-substrate for glutathione peroxidase, and a cofactor for various enzymes [54, 55]. A significant decrease in this non-enzymatic antioxidant increases susceptibility to oxidative stress [56, 57]. When utilized to alleviate oxidative stress, there is a possibility that GSH levels may decrease in diabetic rats [58]. Lima et al. [47] reported that in rats treated with Galactomannan GM-DR, GSH levels increased by 25% on the 2nd day and 50% on the 5th day compared to controls administered saline, although no change was observed on the 7th day. Similarly, Assar et al. [50] demonstrated a significant increase in GSH content in rats treated with licorice extract, indicating an improved antioxidant status compared to untreated controls. These findings align closely with our results, as the GSH level in our study mirrored the CAT levels noted by Aneesha et al. [49], who found significantly elevated GSH levels in diabetic wounds treated with bilirubin-deferoxamine on the 3rd, 14th, and 19th days compared to the control group. Conversely, Kant et al. [48] investigated the effects of quercetin in a diabetic rat wound model and reported no significant differences in GSH levels across groups, except on day 14, where an increase was noted [48]. These contrasting findings emphasize the variability in GSH response depending on the treatment and underline the importance of maintaining adequate GSH levels to counteract oxidative stress during wound healing.

Metformin not only regulates glucose levels but also draws attention with its oxidative stress reducing effects. In recent studies, the antioxidative properties of metformin have been emphasised and these properties have been shown to strengthen cellular defence mechanisms. In recent years, the effects of systemic metformin on antioxidant enzymes and oxidative stress markers have been investigated in rodents with hyperglycaemia [59], diabetic and hepatocellular carcinoma (HCC) [60], diabetic kidney [61] and non-alcoholic fatty liver disease (NAFLD) [62] models. All these studies reveal that metformin both helps to alleviate the oxidative stress burden and is effective in maintaining cellular redox balance by increasing antioxidant activity. Thus, organ damage can be reversed. Although the current literature shows that metformin can regulate inflammatory processes by suppressing important markers of oxidative stress, the specific oxidative stress and antioxidant mechanisms in wound healing are still not fully clarified. These findings are consistent with the results of our study and show that topical application of metformin is effective in reducing oxidative stress and increasing antioxidant levels in diabetic wounds and fill this gap in the literature. In particular, the decrease in MDA levels, an important component of oxidative stress, and the increase in antioxidant enzymes confirm that metformin shows a protective effect against oxidative damage. While this study focused on CAT, GSH, and MDA, further studies are needed to evaluate the potential effects of metformin on other enzymes not directly involved in wound healing.

Studies on the effect of metformin on diabetic wounds have gained momentum in recent years, and its role on oxidative stress in particular has attracted great attention. However, there are still points to be clarified on this subject. Our study makes a significant contribution to this gap by focusing on the role of metformin in reducing oxidative stress in diabetic wounds. In particular, topical metformin application can increase antioxidant capacity in wounded tissues while avoiding systemic side effects, and this innovative approach makes a significant contribution to the literature. In addition, a new contribution of our study is that we present the effect of metformin on wound healing not only through biochemical parameters, but also by taking into account oxidative stress load. This allows us to better understand how metformin modulates not only glucose levels but also cellular stress responses. Although it is known in the literature that metformin accelerates wound healing, our study fills an important gap in this field by addressing in detail its effects on oxidative stress and antioxidant defense mechanisms.

Streptozotocin-induced diabetes is one of many factors that cause delayed wound healing because it increases oxidative stress at the wound site. In our previous study, we evaluated the wound healing process through macroscopic assessment and demonstrated that treatment with metformin resulted in faster healing in diabetic groups [31]. Our present study showed that topically applied metformin significantly decreased MDA and TOS levels in wounds, especially diabetic wounds, while it significantly increased the levels of enzymatic or non-enzymatic antioxidants such as TAS, glutathione, and catalase (Fig. 2). Metformin scavenges free radicals, which are more prevalent in the wounds of diabetic rats than in healthy tissue, increasing antioxidant activity at these sites. In conclusion, the results of our current study show that topical metformin application supports wound healing by increasing the capacity of antioxidants, especially in diabetic wounds, with the reduction of oxidants. Large-scale clinical trials of metformin are necessary before they can be recommended.

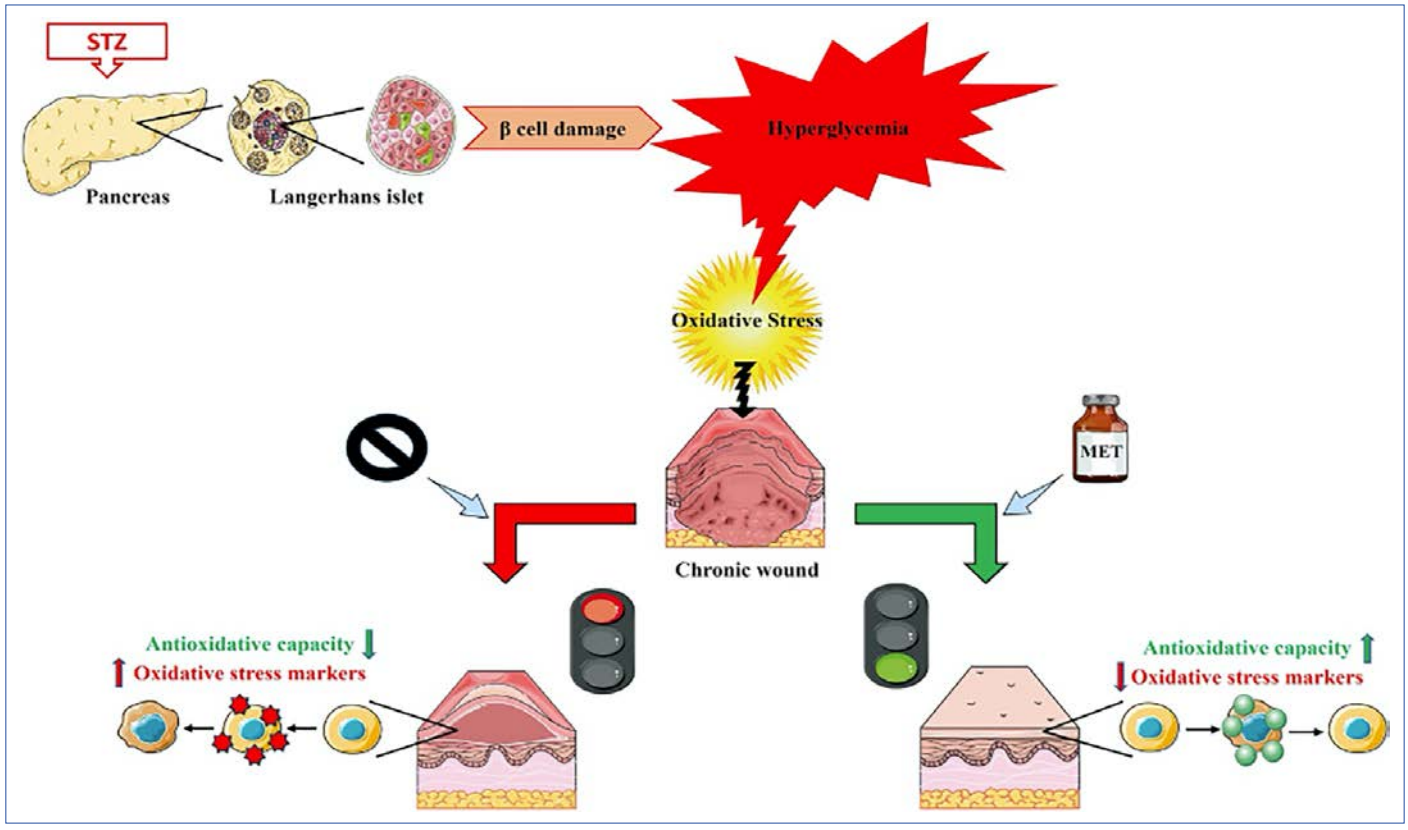

Figure 2. As a result of creating a full-thickness excisional wound model in rats in which we induced diabetes with STZ and treated them with topical metformin for fourteen days, we showed that topical metformin application increased antioxidant capacity by reducing oxidative stress biomarkers in the biopsy samples taken on days 0, 3, 7 and 14, thus improving the wound healing process.

STZ: Streptozotocin; MET: Metformin.

Ethics Committee Approval: The study was approved by The Bezmialem Vakif University Animal Experiments Local Ethics Committee (No: E.69264, Date: 06/07/2022).

Authorship Contributions: Concept – F.K.T., H.D.D.; Design – F.K.T., H.D.D.; Supervision – F.K.T., H.D.D.; Funding – H.D.D.; Materials – F.K.T.; Data collection &/or processing – F.K.T., H.D.D.; Analysis and/or interpretation – F.K.T., H.D.D.; Writing – F.K.T.; Critical review – F.K.T., H.D.D.

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