Metformin Accelerates Wound Healing in Wistar Albino Rats: Evidence from an Excision Wound Model

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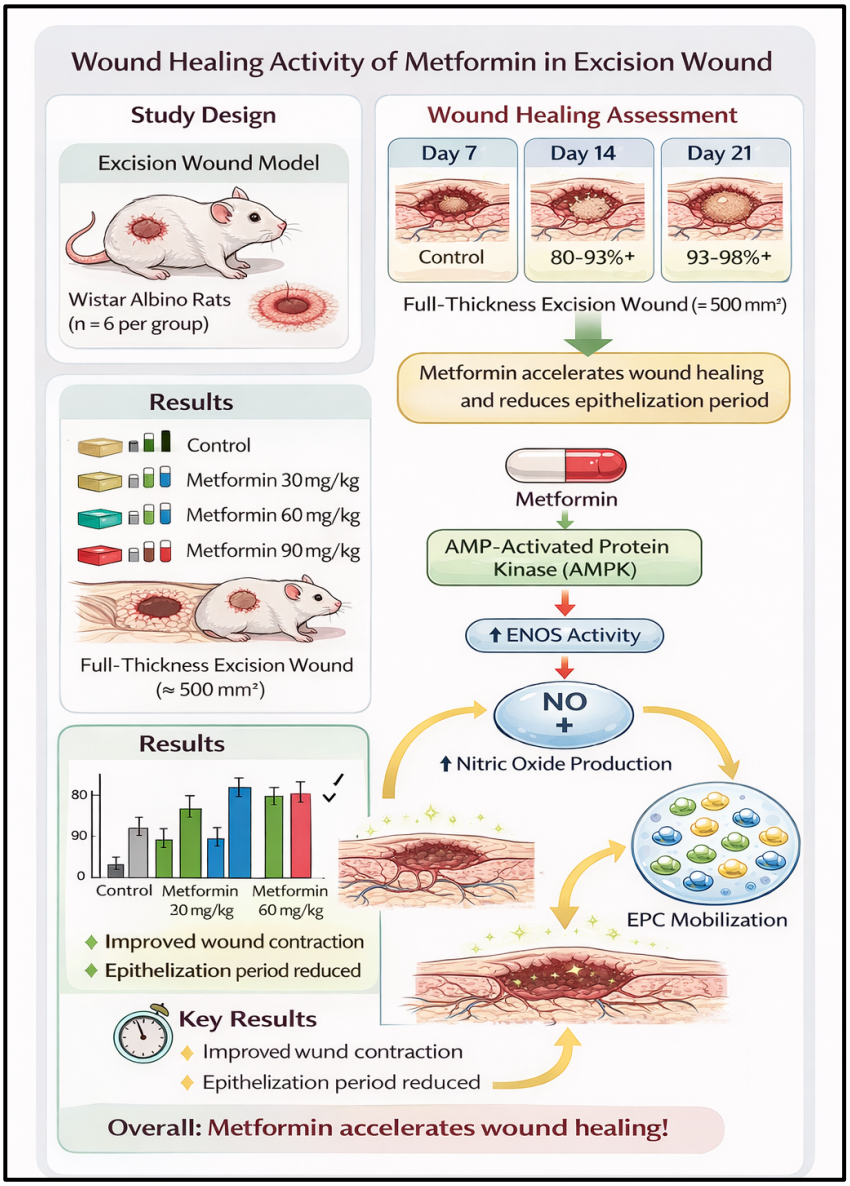

Metformin accelerates wound healing in an excision wound model in Wistar albino rats. Treatment enhanced wound contraction and reduced the epithelization period. The proposed mechanism involves activation of AMPK, increased eNOS activity and nitric oxide production, promoting angiogenesis and endothelial progenitor cell mobilization leading to improved tissue regeneration

Abstract:

Background: Wound healing is a complicated physiological procedure that incorporates inflammation, proliferation and remodelling of tissue. Metabolic disorders and vascular dysfunction are usually linked to impaired wound healing. Metformin is a commonly used biguanide used in the management of type 2 diabetes mellitus, which has been described to have a number of pleiotropic effects other than the glycemic control such as the ability to enhance endothelial function and the angiogenesis process.

Objective: The present study was aimed to evaluate the wound healing activity of metformin in Wistar albino rats using an excision wound model.

Methods: Twenty-four (180 +- 20 g) Wistar albino rats were randomly split into four groups (n = 6). Group I was the control which was given normal saline, and Groups II, III and IV were given metformin 30 mg/kg, 60 mg/kg and 90mg/kg, respectively. Each rat had an incision wound on the back that was about 500 mm2 in diameter and was made through the Morton and Malone method. On days 7, 14 and 21 post wounding, wound contraction was recorded. The time of the epithelization was noted as well. Data were expressed as mean ± SEM and analyzed using one-way ANOVA.

Results: Metformin treatment significantly enhanced wound contraction compared with the control group ($p < 0.05$) and reduced epithelization period. On day 7, wound closure percentages were 32.87 ± 10.42 in the control group and 79.52 ± 11.92, 81.42 ± 9.41, and 93.94 ± 3.72 in the metformin-treated groups (30, 60, and 90 mg/kg respectively). By day 14, wound closure increased to 93.36 ± 2.20, 95.80 ± 2.31, and 98.60 ± 0.47 in the treatment groups. The epithelization period was significantly reduced in metformin-treated animals (11–12.8 days) compared with control animals (20 days).

Conclusion: Metformin significantly accelerates wound healing by enhancing wound contraction and reducing the epithelization period in rats. These findings suggest that metformin may promote tissue repair through mechanisms involving improved endothelial function, enhanced angiogenesis, and activation of AMPK-mediated signaling pathways.

Keywords: Metformin, wound healing, angiogenesis, excision wound model, AMPK, endothelial progenitor cells

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1. Introduction

Wound healing represents a very organized biological process which involves the restoration of the structural and functional integrity of the damaged tissue. It consists of consecutive overlapping stages such as inflammation, proliferation, and remodelling. These phases can be described as the inflammatory cell recruitment, fibroblast expansion, extracellular matrix deposition, angiogenesis, and re-epithelialization, all of which provide contribution to the process of tissue regeneration and normal tissue architecture restoration[1,2].

Delayed/impaired wound healing is one of the greatest clinical problems on a global scale and is often linked to metabolic diseases like diabetes mellitus. Chronic hyperglycemia triggers a number of pathophysiological processes such as endothelial dysfunction, oxidative stress, angiogenic impairment, and diminished nitric oxide bioavailability. The changes interfere with the normal wound healing cascade and could lead to chronic non-healing wounds, especially diabetic foot ulcers[3,4]. Chronic wounds have a significant financial impact on the healthcare systems and are linked to higher morbidity, risk of infection, and amputation of the limb.

Metformin is a derivative of biguanide which is commonly administered as pharmacological therapy in the management of type 2 diabetes mellitus. In addition to the glucose-lowering effect, metformin has received interest on its pleiotropic pharmacological effects, such as the endothelial functionality enhancement, anti-inflammatory effects, and enrichment of vascular repair mechanisms[5,6]. AMP-activated protein kinase (AMPK) activation is one of the primary mechanisms that cause these effects, and the intracellular energy sensor that is essential in the process of cellular metabolism and endothelial homeostasis regulation.

AMPK Finding AMPK has been found to be activated by metformin and induce endothelial nitric oxide synthase (eNOS) leading to increased production of nitric oxide and vascular functions. In angiogenesis and tissue repair, nitric oxide is an important cytokine that allows vasodilation, improving blood flow, and endothelial cell growth and migration[7]. Increased angiogenesis will guarantee sufficient oxygen and nutrient supply to the injured tissues and thus the wound will heal faster.

Metformin has also been reported to stimulate bone marrow-derived endothelial progenitor cells (EPCs) growth and activities besides its vascular protection properties. EPCs play an important role in neovascularization and endothelial repair after tissue damage, and mobilization of them has been linked with better wound healing results[4,8]. Experimental research has established that angiogenic activity, as well as tissue regeneration is improved by metformin treatment in diabetic animal models.

Despite these promising observations, limited experimental studies have investigated the direct effect of metformin on wound healing under non-diabetic

conditions. Understanding the potential role of metformin in promoting tissue repair could provide valuable insights into its therapeutic applications beyond glycemic control. Therefore, the present study was designed to evaluate the wound healing activity of metformin in Wistar albino rats using an excision wound model.

2. Materials and Methods

2.1 Experimental Animals

The present experimental study was conducted using healthy adult male Wistar albino rats weighing 180 ± 20 g. The animals were procured from the Central Animal House facility of Bharati Vidyapeeth (Deemed to be University) Medical College, Sangli, India. Animals were acclimatized for one week prior to experimentation under standard laboratory conditions.

The rats were housed in polypropylene cages with stainless steel grid tops and maintained under controlled environmental conditions with a temperature of 25 ± 2°C, relative humidity of 55–65%, and a 12-hour light–dark cycle. Animals were provided with a standard pellet diet and water ad libitum throughout the study period.

All experimental procedures were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) prior to commencement of the experiment (Approval No.: IAEC/2017/03).

2.2 Chemicals and Drugs

Metformin tablets (250 mg) were obtained from a certified pharmaceutical supplier and used as the test drug in the present study. Normal saline (0.9% NaCl) was used as the vehicle control for drug administration. Thiopental sodium was used as the anesthetic agent during the surgical procedure for wound creation. Surgical procedures were performed using sterile instruments including scalpel blades, surgical scissors, artery forceps, cotton, and sterile gauze.

All chemicals and reagents used in the study were of analytical grade and were prepared freshly prior to use.

2.3 Experimental Design

The animals were randomly divided into four experimental groups, with six rats in each group (n = 6).

Group I (Control): Animals received normal saline and served as the control group.

Group II (Metformin 30 mg/kg): Animals received metformin at a dose of 30 mg/kg body weight orally once daily.

Group III (Metformin 60 mg/kg): Animals received metformin at a dose of 60 mg/kg body weight orally once daily.

Group IV (Metformin 90 mg/kg): Animals received metformin at a dose of 90 mg/kg body weight orally once daily.

Metformin was dissolved in normal saline and administered using an oral feeding needle throughout the experimental period.

The doses of metformin used in this study were selected based on previous experimental studies and human-to-animal dose conversion methods[9,10].

2.4 Excision Wound Model

The wound healing activity of metformin was evaluated using the excision wound model described by Morton and Malone, a widely accepted experimental method for assessing wound healing potential in laboratory animals[11].

Prior to the surgical procedure, the animals were fasted overnight with free access to water. On the day of the experiment, rats were anesthetized with thiopental sodium (25 mg/kg, intraperitoneally) to ensure adequate anaesthesia during wound creation.

The dorsal thoracic region of each rat was carefully shaved using electric clippers and disinfected with 70% ethanol to maintain aseptic conditions. A full-thickness circular excision wound of approximately 500 mm² was created on the shaved dorsal surface using a sterile scalpel blade.

The wounds were left undressed and open throughout the experimental period to allow natural healing. Following wound creation, animals were housed individually in separate cages to prevent wound contamination, biting, or interference by other animals.

2.5 Evaluation of Wound Healing Parameters

The wound healing activity was assessed by measuring percentage wound contraction and period of epithelization during the experimental period.

2.5.1 Measurement of Wound Contraction

Wound healing progression was monitored by measuring the wound area on days 7, 14, and 21 post-wounding. The wound margins were traced on transparent polythene sheets, and the traced area was subsequently transferred onto millimeter graph paper to determine the wound size.

The percentage of wound contraction was calculated using the following formula:

$$\text{Percentage wound contraction} = (A_0 - A\_d) / A_0 \times 100$$

Where:

A_0 = Initial wound area on day 0

Ad = Wound area on the respective day of measurement

The rate of wound contraction was used as an indicator of the wound healing process.

2.5.2 Period of Epithelization

The epithelization period was recorded as the number of days required for complete wound closure, indicated by the formation and subsequent shedding of the scab (eschar) without any residual raw wound area.

This parameter reflects the time required for complete epithelial regeneration and was recorded individually for each experimental animal.

2.6 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare differences among the experimental groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of Metformin on Percentage Wound Closure

The effect of metformin on wound healing was evaluated by measuring the percentage of wound contraction on days 7, 14, and 21 following wound creation.

Table 1. Percentage wound closure on day 7

Group	Treatment	Wound Closure (%)
I	Control	32.87 ± 10.42
II	Metformin 30 mg/kg	79.52 ± 11.92
III	Metformin 60 mg/kg	81.42 ± 9.41
IV	Metformin 90 mg/kg	93.94 ± 3.72

Values are expressed as mean ± SEM (n = 6).

On day 7, animals treated with metformin exhibited significantly higher wound contraction compared with the control group ($p < 0.05$). The highest percentage of wound closure was observed in the metformin 90 mg/kg group, indicating a pronounced improvement in the early phase of wound healing.

Table 2. Percentage wound closure on day 14

Group	Treatment	Wound Closure (%)
I	Control	83.43 ± 4.60
II	Metformin 30 mg/kg	93.36 ± 2.20
III	Metformin 60 mg/kg	95.80 ± 2.31
IV	Metformin 90 mg/kg	98.60 ± 0.47

Values are expressed as mean ± SEM (n = 6).

By day 14, wound contraction increased in all groups; however, metformin-treated animals continued to demonstrate greater wound closure compared with control animals. The 90 mg/kg metformin group showed the highest wound contraction, suggesting enhanced progression of the healing process.

Table 3. Percentage wound closure on day 21

Group	Treatment	Wound Closure (%)
I	Control	96.79 ± 2.60
II	Metformin 30 mg/kg	97.64 ± 0.91
III	Metformin 60 mg/kg	98.92 ± 0.59
IV	Metformin 90 mg/kg	99.60 ± 0.14

Values are expressed as mean ± SEM (n = 6).

On day 21, wound healing was nearly complete in all groups. Although the metformin-treated groups showed slightly higher wound contraction compared with the control group, the differences were minimal, indicating completion of the natural healing process.

3.2 Effect of Metformin on Epithelization Period

The epithelization period, defined as the number of days required for complete wound closure with shedding of the eschar, was recorded for each group.

Table 4. Period of Epithelization

Group	Treatment	Epithelization Period (days)
I	Control	20.0 ± 0.0
II	Metformin 30 mg/kg	11.5 ± 0.2
III	Metformin 60 mg/kg	12.83 ± 0.31
IV	Metformin 90 mg/kg	11.0 ± 0.45

Values are expressed as mean ± SEM (n = 6).

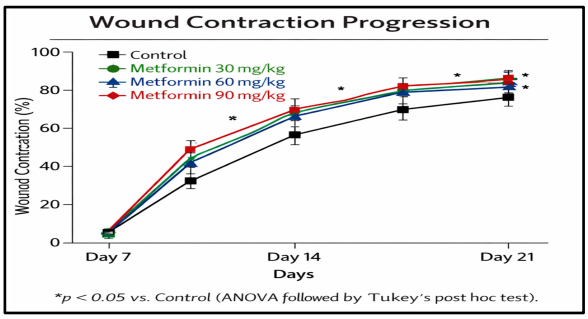

Figure 1. Progression of wound contraction in the excision wound model

The percentage of wound contraction was measured on Days 7, 14, and 21 following wound creation in Wistar albino rats. Animals treated with metformin (30, 60, and 90 mg/kg) showed significantly enhanced wound closure compared with the control group. The highest rate of wound contraction was observed in the metformin 90 mg/kg group during the early phase of healing. Data are expressed as mean ± SEM (n = 6). $p < 0.05$ vs. control (one-way ANOVA followed by Tukey's post-hoc test).

Metformin-treated groups showed a significant reduction in the epithelization period compared with the control group ($p < 0.05$). The shortest epithelization period was observed in the metformin 90 mg/kg group, indicating faster regeneration of epithelial tissue and enhanced wound healing.

4. Discussion

The present study was conducted to evaluate the wound healing activity of metformin using an excision wound model in Wistar albino rats. The results demonstrated that metformin treatment significantly accelerated wound contraction and reduced the epithelization period compared with the control group. These findings indicate that metformin may possess significant wound healing potential beyond its conventional role as an antidiabetic drug.

Wound healing is a complex physiological process involving a series of coordinated events including inflammation, proliferation, and tissue remodeling. These processes involve cellular migration, fibroblast proliferation, extracellular matrix formation, collagen synthesis, angiogenesis, and epithelial regeneration[12]. Any disturbance in these processes may lead to delayed or impaired wound healing.

In the present study, metformin-treated animals exhibited significantly higher wound contraction on day 7 and day 14 compared with control animals. Early wound contraction is a critical indicator of tissue repair and is primarily mediated by fibroblast activity, collagen deposition, and granulation tissue formation[13]. The enhanced wound contraction observed in metformin-treated groups suggests that metformin may stimulate fibroblast proliferation and collagen synthesis during the early phases of wound healing.

Angiogenesis plays a vital role in the wound healing process by supplying oxygen and nutrients to regenerating tissues. Adequate vascularization is essential for maintaining cellular metabolism and supporting tissue repair. Previous studies have shown that metformin improves endothelial function and enhances angiogenesis through activation of AMP-activated protein kinase (AMPK) signaling pathways[14]. Activation of AMPK promotes endothelial nitric oxide synthase (eNOS) activity, leading to increased nitric oxide production and improved microvascular circulation.

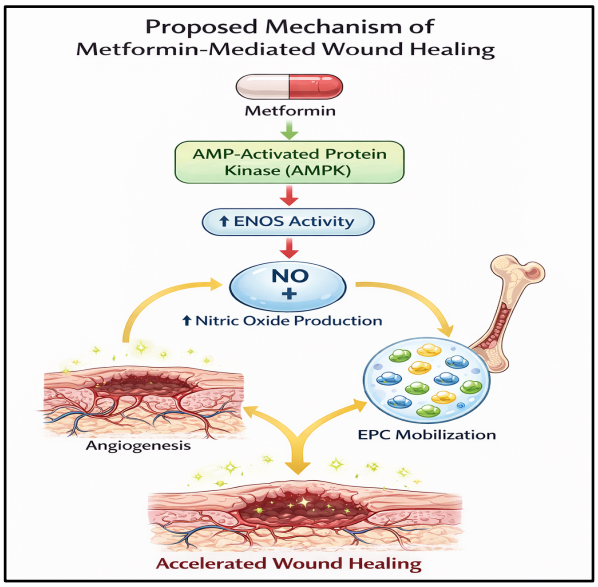

Figure 2. Proposed mechanism of metformin-mediated wound healing

Metformin activates AMP-activated protein kinase (AMPK), which enhances endothelial nitric oxide synthase (eNOS) activity and increases nitric oxide production. Increased nitric oxide promotes angiogenesis and mobilization of endothelial progenitor cells (EPCs), leading to accelerated wound healing.

Nitric oxide is a key mediator involved in vascular homeostasis and tissue regeneration. It promotes vasodilation, enhances blood flow, and facilitates endothelial cell migration and proliferation, all of which contribute to improved wound healing[15]. In the present study, the improved wound contraction observed in metformin-treated groups may be attributed to increased nitric oxide production resulting from AMPK activation.

In addition to improving endothelial function, metformin has been reported to enhance the mobilization of endothelial progenitor cells (EPCs) derived from bone marrow. EPCs play a crucial role in neovascularization and endothelial repair following tissue injury[16]. Increased mobilization of EPCs contributes to improved vascular regeneration and enhanced tissue repair. Experimental studies have demonstrated that metformin treatment increases circulating EPC levels and improves their functional activity[17].

Furthermore, metformin has been reported to possess anti-inflammatory properties that may contribute to improved wound healing. Inflammation is an essential early phase of wound repair; however, prolonged or excessive inflammation may impair tissue regeneration[18]. Metformin has been shown to inhibit inflammatory signaling pathways, including nuclear factor kappa B (NF-κB), thereby reducing the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [19]. Suppression of excessive inflammatory responses may facilitate a favorable environment for tissue repair and regeneration.

Another important parameter evaluated in the present study was the epithelization period, which represents the time required for complete epithelial coverage of the wound surface. The results demonstrated that metformin-treated animals exhibited a significantly shorter epithelization period compared with the control group. Accelerated epithelial regeneration indicates enhanced keratinocyte proliferation and migration during the wound healing process [20].

The observed reduction in epithelization time in metformin-treated groups suggests that metformin may promote epithelial cell proliferation and differentiation. This effect may be mediated through improved vascular supply, enhanced cellular metabolism, and increased growth factor activity during the proliferative phase of wound healing.

Several previous experimental studies support the wound healing potential of metformin. Han et al. demonstrated that metformin accelerated wound healing in diabetic mice by activating the AMPK/eNOS signaling pathway and improving angiogenesis[8]. Similarly, other studies have reported that metformin enhances endothelial function, reduces oxidative stress, and improves tissue regeneration following injury (5,6).

In the present study, the highest wound contraction was observed in the metformin 90 mg/kg group, suggesting a dose-dependent improvement in wound healing parameters during the early phase of healing. However, by day 21, wound closure was nearly complete in all groups, indicating that the natural healing process eventually progressed in both treated and control animals.

The findings of the present study suggest that metformin may accelerate wound healing through multiple mechanisms, including activation of AMPK signaling pathways, increased nitric oxide production, enhanced angiogenesis, mobilization of endothelial progenitor cells, and modulation of inflammatory responses.

Despite these promising findings, certain limitations should be considered. The study was conducted in healthy non-diabetic rats, and therefore the results may not fully represent the complex pathophysiological conditions present in diabetic wounds. Further studies involving diabetic animal models and molecular investigations are required to better understand the underlying mechanisms responsible for metformin-induced wound healing.

Overall, the present study provides experimental evidence supporting the potential role of metformin in promoting wound healing and tissue regeneration

5. Conclusion

The present experimental study demonstrated that metformin significantly enhances wound healing in Wistar albino rats using the excision wound model. Metformin-treated groups showed accelerated wound contraction and a markedly reduced epithelization period compared with the control group. These findings indicate that metformin promotes faster tissue repair and epithelial regeneration.

The improved wound healing observed in the present study may be attributed to multiple mechanisms, including activation of AMP-activated protein kinase (AMPK), increased nitric oxide production, enhanced angiogenesis, and improved endothelial function. In addition, metformin may facilitate mobilization of endothelial progenitor cells and modulate inflammatory responses, thereby promoting an optimal environment for tissue regeneration.

Overall, the results suggest that metformin possesses potential therapeutic benefits in promoting wound healing beyond its conventional role as an antihyperglycemic agent. Further studies involving diabetic wound models and molecular investigations are required to elucidate the precise mechanisms underlying its wound healing activity and to explore its possible clinical applications in wound management.

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Conflict of Interest
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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