

Therapeutic Effects of Cilnidipine (1%) Gel on Ischemia-Induced Ulcers: A Rabbit Ear Model Study

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ABSTRACT

Background: Venous ischemic ulcers are a chronic and recurrent health concern affecting 1-2% of the adult population worldwide. While the pathophysiology of chronic ischemic wounds remains complex and not fully understood, persistent inflammation is believed to be a key contributing factor. Pentoxifylline, a dimethyl xanthine derivative, is widely used for treating peripheral vascular diseases, whereas Cilnidipine, a fourth-generation calcium channel blocker, has vasodilatory and anti-inflammatory properties and is primarily used for managing hypertension. **Objective:** This study aims to evaluate the therapeutic effects of Cilnidipine (1%) gel on ischemic ulcers using a rabbit ear model. **Methods:** A total of 40 domestic rabbits (both male and female, weighing 1.5–2 kg) were randomly assigned into four experimental groups (10 rabbits per group). The induction group underwent ischemia by ligation of the rostral and central arteries and veins at the base of the ear for 14 days without treatment. The control group received a vehicle gel applied topically for 30 days post-ischemia induction. The pentoxifylline group was treated with pentoxifylline gel (1%) twice daily for 14 days following ischemia induction. The cilnidipine group was treated with cilnidipine gel (1%) twice daily for 14 days following ischemia induction. Tissue homogenates and serum samples were analyzed to assess interleukin-6 (IL-6), matrix metalloproteinase-9 (MMP-9), tumor necrosis factor-alpha (TNF- α), and vascular endothelial growth factor-A (VEGF-A). Additionally, histological evaluations of ear tissue sections were conducted. **Results:** The induced non-treated ischemic group exhibited significantly elevated levels of IL-6, TNF- α , and MMP-9, while VEGF-A levels were significantly decreased compared to the healthy control group. Treatment with cilnidipine gel (1%) significantly reduced IL-6, TNF- α , and MMP-9 levels, while VEGF-A levels were significantly increased compared to the induction group. Histopathological scoring showed marked improvements in the cilnidipine-treated group, with significant angiogenesis and reduced inflammatory damage. No significant difference in histopathological improvement was observed between the pentoxifylline and cilnidipine groups. **Conclusion:** Cilnidipine gel (1%) applied twice daily effectively enhanced ischemic wound healing by reducing inflammation, oxidative tissue damage, and increasing angiogenic activity. These findings suggest that cilnidipine may be a promising therapeutic agent for ischemic ulcer management.

Keywords: Cilnidipine, Ischemia, TNF- α , Inflammatory Cytokines, Rabbits.

INTRODUCTION

Hypoxia is inevitable in wounded tissue, and oxygen delivery to the wound site is essential to promote healing. Energy is required for intracellular functions like biosynthesis, movement, and transport. This energy is provided by adenosine triphosphate coenzyme (ATP). Oxid phosphorylation produces ATP in mitochondria and is the most critical molecular/enzymatic reservoir of chemical energy. This oxidative chain reaction is oxygen-dependent; it cannot occur without oxygen. The processes of aerobic glycolysis, the oxidation of fatty acids, and the citric acid cycle are closely linked to energy generation through oxidative phosphorylation and are oxygen-dependent [1]. Cellular injury often results from ischemia. Anaerobic glycolysis maintains the production of energy in contrast to hypoxia. After reducing potential substrates, anaerobic energy production stops in tissues suffering from ischemia; therefore, ischemia causes more severe and rapid tissue damage than hypoxia. Every chronic wound begins as an acute wound, but instead of healing normally, it becomes stuck in a longer inflammatory phase. Specifically, there is an increase of the cytokines IL-6, IL-1, and TNF- α at this stage, as well as elevated levels of matrix metalloproteinases (MMPs) [2]. Cilnidipine is the fourth generation of calcium channel blockers (CCB). It is a dual calcium channel blocker, which blocks both L-type voltage-gated calcium channels in vascular smooth muscle and N-type calcium channels in sympathetic nerve terminals, which supply blood vessels. Cilnidipine enhances nitric oxide generation by enhancing endothelial nitric oxide synthase and relaxes human arteries by calcium channel

antagonistic action [3]. The study aimed to evaluate the effects of topical applications of cilnidipine in treating ischemia-induced ulcers in rabbit ear models.

MATERIALS AND METHODS

Animal Model and Experimental Design

The institutional review board (IRB) in the College of Medicine/Al-Nahrain University approved the study protocol with the principles of laboratory animal care. In this research, 40 young adult, apparently healthy New Zealand white rabbits of males and females weighing (1.5-2) kg were divided into four experimental groups, each with ten animals. The study lasted from the beginning of October 2022 until the end of March 2023, when the animals were housed in animal houses and allowed free access to water and food under controlled temperature conditions. Before the start of the study, the animals were separated into groups and left in separate cages for ten days to acclimate to the animal house environment.

Induction of Ischemic Skin Ulcer in Rabbit Ear

The surgical methods used to induce ischemic wounds were taken from a modified strategy outlined by [4, 5]. The rabbits were kept on a suitable pad for all surgeries, carried out in sterile conditions, with the surgical regions cleaned with ethanol. The dorsal and ventral sides of the right ear and the regions around the ear base were shaved before

the production of anesthesia by the administration of ketamine (50mg/kg - intramuscular) and xylazine (5mg/kg - intramuscular) injections. Additionally, cefotaxime (100 mg/kg - intramuscular) was administered to reduce the risk of infections. Subsequently, a diluted lidocaine without epinephrine (7mg/kg) was given subcutaneously into the ear as a local anesthetic. The rostral and central arteries and the rostral and central veins were ligated using silk sutures (4/0) to induce ischemia at two specific locations at the base of the ear. This surgical procedure ensured that the minor caudal artery and vein remained the sole sources of blood supply to the ear without incisions in the ear tissues [6]. Following surgery, rabbits were given free access to food and water, and cefotaxime (100 mg/kg), intramuscular, was administered once daily for three days to protect against infection with staphylococcus aureus, enterococcus faecium, and pseudomonas aeruginosa [7]. Sutures were removed after two weeks of daily application of fusidic acid cream 2% and twice-day administration of cefixime 100 mg suspension with paracetamol suspension for pain relief. On day 30, the rabbits were subjected to anesthesia by the established protocol. Subsequently, a skin biopsy was performed on the rabbit's ear using a 10 mm punch biopsy tool. The biopsy was inflicted on the anterior ear region, which had a diameter of 1 cm. Specifically, 3-4 horizontal sites were prepared for obtaining tissue homogenate and histopathological analysis [2].

The Protocol for the Experiments in this Study

Control group: Topical vehicle gel was applied to the left ear of apparently healthy rabbits for 30 days. Induction group: After 30 days of topical application of vehicle gel, a cardiac puncture was used to collect blood samples. A skin biopsy was taken from the right rabbit's ears for tissue homogenate and histology preparation. Pentoxifylline gel (1%) group: After inducing ischemia, right rabbits' ears received pentoxifylline gel (1%) topically on day 16 twice daily for 14 days. On day 30, a cardiac puncture was used to collect blood samples, and a skin biopsy was taken from the right rabbits' ears for tissue homogenate and histology preparation. Cilnidipine gel (1%) group: After inducing ischemia, right rabbits' ears received cilnidipine gel (1%) topically on day 16 twice daily for 14 days. On day 30, a cardiac puncture was used to collect blood samples, and a skin biopsy was taken from the right rabbits' ears for tissue homogenate and histology

preparation.

Samples Preparation (Serum and Tissue Homogenate)

After anesthetizing the animals with ketamine (50mg/kg) and xylazine (5mg/kg), intramuscular injections and diluted lidocaine (7mg/kg S.C.) were administered in the ear. Blood was obtained from the animals via intra-cardiac puncture and placed in a serum separator tube (a plastic gel tube without an anticoagulant), where it was left to clot for two hours at room temperature before being centrifuged for 20 minutes at around 3,000 rpm.

Punch biopsies (10 mm) were used to cut off tiny fragments of the ear, and the resulting tissues were soaked in ice-cold phosphate-buffered saline (PBS) (0.01mol/L, pH 7.0-7.4) at 4 C to remove excess blood, weighed and homogenized. The homogenates were then centrifuged at 5000 rpm. for 5 minutes. The ELISA technique (enzyme-linked immune sorbent assay) was used in this kit. Assaying IL-6, TNF- α , MMP-9, and VEGF-A in rabbit serum, plasma, and other relevant biological materials relied on a test concept based on the biotin double antibody sandwich technique [8-11].

Histopathological Sample Preparation

Using a punch biopsy (10 mm), tissue samples from the ears were cut and preserved in a 10% formalin solution for at least 24 hours. The specimen was dehydrated in ethanol at 70%, 80%, 95%, and 100% for 2 hours at each concentration. Before being dipped in liquid paraffin at 55-60C, the specimen was cleaned with xylol (2 stages, 2 hours each). Tissue was put into hard paraffin wax, heated to 56-58 C, and poured into the models. This made paraffin blocks. Sections of 5 μ m thickness were cut from blocks using a microtome, transferred with forceps to a water bath at 45 C, and placed on glass slides. Tissue sections were dewaxed in an oven at 55 C. They were examined with a light microscope after hematoxylin and eosin staining [12].

Histological Scoring System

Histopathological changes in both ears of each rabbit were scored using a histological scoring system based on the quality and quantity of healing, such as granulation tissue formation, the degree of re-epithelialization, angiogenesis, and the presence of inflammatory cells [13, 14].

Table 1: Histopathological Scoring System

Score No.	Re-Epithelialization	Granulation Tissue	Inflammatory Cells	Angiogenesis
0	Absence of epithelial Proliferation $\geq$ 70% of the tissue	Immature and inflammatory tissue in $\geq$ 70% of the tissue	13 -15 cells/filed	Absence of angiogenesis ,presence of congestion, with edema
1	Poor epidermal organization in $\geq$ 60% of the tissue	Thin immature and inflammatory tissue in $\geq$ 60% of the tissue	13-10 cells/filed	1-2 vessels/site, Hemorrhage congestion, with edema
2	Incomplete epidermal organization in $\geq$ 40% of the tissue	Moderate remodeling in $\geq$ 40% of the tissue	7-10 cells/filed	3-4 vessels/site, moderate edema and congestion
3	Moderate epithelial proliferation in $\geq$ 60% of the tissue	Thick granulation layer and well-formed collagen matrix $\geq$ 60% of the tissue	4-7 cells/filed	5-6 vessels/site slight edema and congestion,
4	Complete epithelial remodeling in $\geq$ 80% of the tissue	Complete tissue organization in $\geq$ 80% of the tissue	1-4 cells/filed	More than 7 vessels/site vertically disposed toward the epithelial surface
5	Normal epidermal and dermal layers with no epithelial proliferation	Normal epithelial thickness with normal skin appendages	1-3 cells/filed	NO

Statistical Analysis

Data entry and analysis were performed using Microsoft Excel 2010 and SPSS version 26. Continuous variables were expressed as mean $\pm$ SD. Categorical variables were presented as frequencies and

percentages using the Chi-square test. Tests of Normality (Kolmogorov Smirnova & Shapiro-Wilk) showed that data were non-normally distributed; thus, non-parametric tests (Mann Whitney & Kruskal Willis) were used instead of parametric tests (independent t-test & one-way ANOVA). The level of significance was considered when the P value

was less than 0.05 [15].

Results

Changes in Serum and Tissue Homogenate Ischemic Parameter Levels in the Induction Group

Serum and tissue homogenate levels of inflammatory mediators (IL-6, TNF- α) and matrix metalloproteinase-9 (MMP-9) significantly increased among the induced non-treated ischemic group compared with an apparently healthy control group ($P < 0.001$). Serum and tissue homogenate levels of the angiogenic stimulator (VEGF-A) were significantly decreased among the induced non-treated ischemic group in comparison with an apparently healthy control group ($P < 0.001$) [Tables (2 and 3), Figures (1 to 4)].

Effects of topical pentoxifylline gel on ischemic parameter (serum and tissue homogenate) levels after induction of ischemia:

Serum and tissue homogenate levels of (TNF- α), (IL-6), and (MMP-9) in rabbits given topical pentoxifylline gel twice daily were

significantly reduced in comparison with an induced non-treated ischemic group ($P < 0.001$). At the same time, serum and tissue homogenate levels of (VEGF-A) were significantly elevated among the pentoxifylline group compared to the induction group ($P < 0.001$) [Tables (2 and 3), Figures (1 to 4)].

Effects of Topical Cilnidipine Gel on Ischemic Parameter (Serum and Tissue Homogenate) Levels After Induction of Ischemia

Serum and tissue homogenate levels of (TNF- α), (IL-6), and (MMP-9) in rabbits given topical cilnidipine gel twice daily were significantly reduced in comparison with an induced non-treated ischemic group ($P < 0.001$), and they showed no statistically significant differences compared to a group given topical pentoxifylline gel. At the same time, serum levels of (VEGF-A) were significantly elevated among the cilnidipine group compared to the pentoxifylline group ($P < 0.001$). Tissue homogenate levels of (VEGF-A) showed no statistically significant differences among the cilnidipine group compared to a group given topical pentoxifylline gel [Tables (2 and 3), Figures (1 to 4)].

Table 2: Comparison Among Tested Groups in the Study Regarding Mean Serum Levels ($\pm$ SD) of (IL-6), (TNF- α), (MMP-9), and (VEGF-A)

Parameters	Groups			
	Control	Induction	Pentoxifylline Gel (1%)	Cilnidipine Gel (1%)
IL-6 (ng/L)	80.9 $\pm$ 8.9	197.1 $\pm$ 33.6 a**	97.5 $\pm$ 11.9 b**	92.2 $\pm$ 11.6 b**, c ^{NS}
TNF-a (ng/L)	775.5 $\pm$ 266.2	2403.1 $\pm$ 805.4 a**	844.6 $\pm$ 94.0 b**	948.8 $\pm$ 248.8 b**, c ^{NS}
MMP-9 (ng/ml)	39.7 $\pm$ 6.7	138.0 $\pm$ 63.8 a**	42.9 $\pm$ 10.9 b**	47.9 $\pm$ 8.5 b**, c ^{NS}
VEGF-A (pg/ml)	65.7 $\pm$ 19.6	37.7 $\pm$ 6.7 a**	72.0 $\pm$ 10.8 b**	92.1 $\pm$ 13.4 b**, c*

IL-6: Interleukin-6; TNF-a: Tumour necrosis factor- α ; MMP-9: Matrix metalloproteinase-9; VEGF-A: Vascular endothelial growth Factor-A. a: Comparison with the control group; b: comparison with the induction group; c: comparison with the pentoxifylline group; *: significant difference ($0.05 \leq P < 0.001$); **: P highly significant difference ($P < 0.01$); NS: no significant difference ($P > 0.05$)

Table 3: Comparison Among Tested Groups in the Study Regarding Mean Tissue Homogenate Levels ($\pm$ SD) of (IL-6), (TNF- α), (MMP-9), and (VEGF-A)

Parameters	Groups			
	Control	Induction	Pentoxifylline Gel (1%)	Cilnidipine Gel (1%)
IL-6 (ng/L)	88.2 $\pm$ 5.2	246.4 $\pm$ 21.0 a**	103.3 $\pm$ 9.5 b**	104.2 $\pm$ 11.7 b**, c ^{NS}
TNF-a (ng/L)	1056.7 $\pm$ 499.7	2880.3 $\pm$ 684.8 a**	1103.2 $\pm$ 154.3 b**	1127.4 $\pm$ 184.0 b**, c ^{NS}
MMP-9 (ng/ml)	53.5 $\pm$ 11.6	233.4 $\pm$ 49.6 a**	69.3 $\pm$ 12.6 b**	67.8 $\pm$ 11.3 b**, c ^{NS}
VEGF-A (pg/ml)	80.5 $\pm$ 8.8	12.5 $\pm$ 42.6 a**	100.1 $\pm$ 13.8 b**	123.0 $\pm$ 37.5 b**, c ^{NS}

IL-6: Interleukin-6; TNF-a: Tumour necrosis factor- α ; MMP-9: Matrix metalloproteinase-9; VEGF-A: Vascular endothelial growth Factor-A. a: Comparison with the control group; b: comparison with the induction group; c: comparison with the pentoxifylline group; *: significant difference ($0.05 \leq P < 0.001$); **: P highly significant difference ($P < 0.01$); NS: no significant difference ($P > 0.05$)

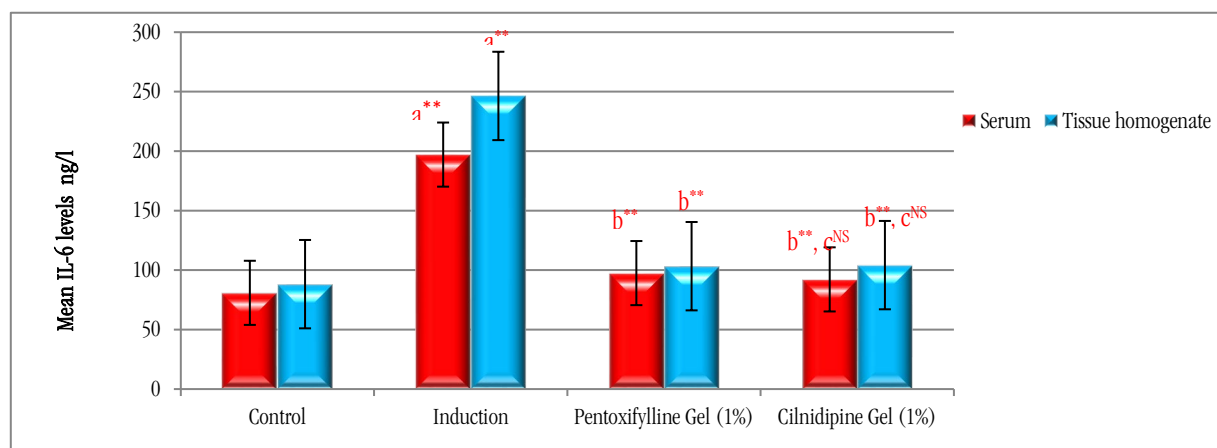
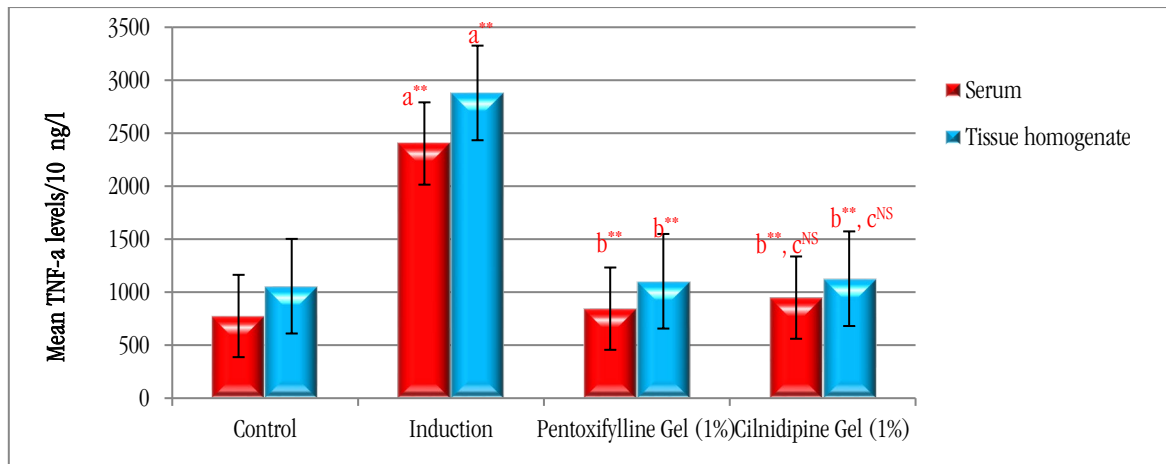
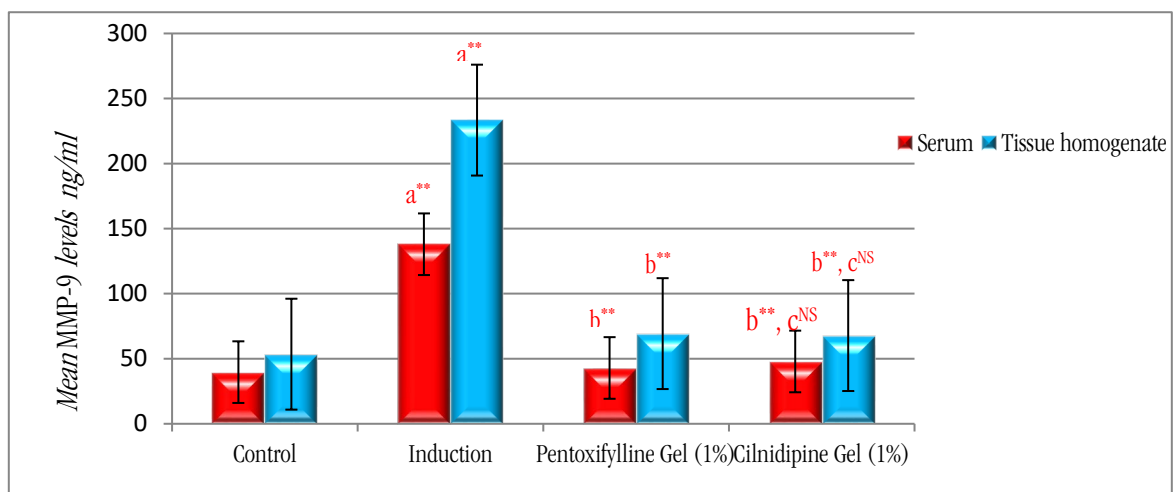


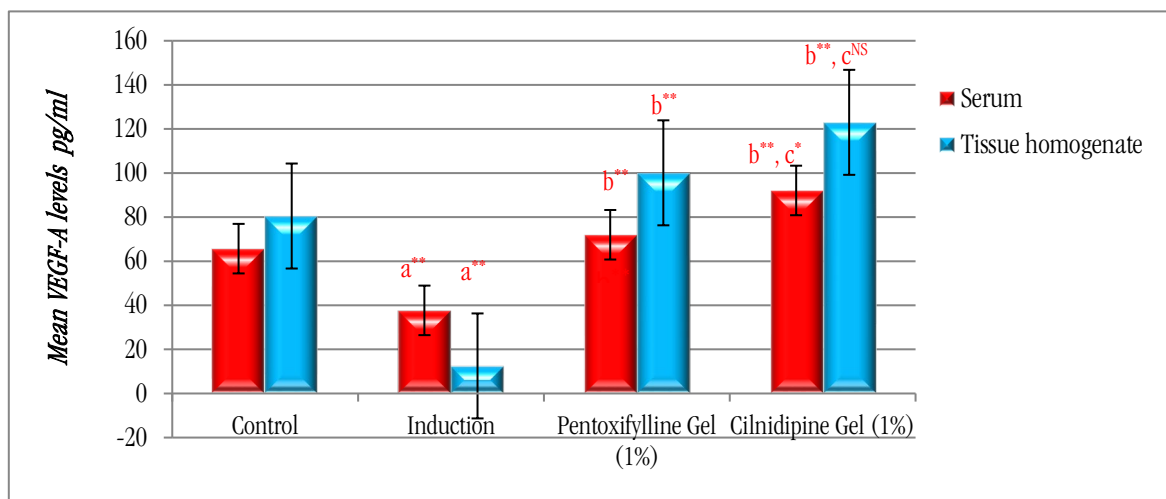
Figure 1: Comparison Among Tested Groups in the Study Regarding Mean Levels of IL-6 In Both Serum and Tissue Homogenate of Rabbit Ear Skin.



TNF-α: Tumour Necrosis Factor-α

FIGURE 2: Comparison Among Tested Groups in the Study Regarding Mean Levels of TNF-α in both Serum and Tissue Homogenate of Rabbit Ear Skin.

MMP-9: Matrix Metalloproteinase-9

FIGURE 3: Comparison Among Tested Groups in the Study Regarding Mean Levels of MMP-9 In Both Serum and Tissue Homogenate of Rabbit Ear Skin.

VEGF-A: Vascular Endothelial Growth Factor-A

FIGURE 4: Comparison Among Tested Groups in the Study Regarding Mean Levels Of VEGF-A In Both Serum and Tissue Homogenate of Rabbit Ear Skin.

Results of Histopathological Evaluation

The histopathological evaluation score after ischemia induction was significantly lower in the induction group (score 1) than in the control group (score 5). The skin section of the induction group shows incomplete epidermal organization and moderate remodeling of the

tissue. The histopathological evaluation score after induction of ischemia was significantly elevated in the pentoxifylline group (score 5) compared to the induction group, both the epidermal layer and the appendage component had a normal appearance. A significant proliferation of fibroblasts and newly formed blood vessels was

observed.

Histopathological evaluation score after induction of ischemia was significantly elevated among the cilnidipine group (score 4) compared to the induction group, a significant proliferation of fibroblasts and newly formed blood vessels was observed. The

epidermal layer and the appendage component had a normal appearance. Comparison between the pentoxifylline and cilnidipine groups regarding score means of histopathological evaluation after induction of ischemia revealed no significant difference among both groups [Tables (4 and 5), Figures (5)].

Table 4: Comparison among tested groups in the study regarding score means ($\pm$ SD) of histopathological evaluation

No.	Groups	Mean Score
1	Control	5 $\pm$ 0.0
2	Induction	1.33 $\pm$ 0.516 a**
3	Pentoxifylline Gel (1%)	4.67 $\pm$ 0.516 b**
4	Cilnidipine Gel (1%)	4.17 $\pm$ 0.408 b**,c ^{NS}

a: Comparison with the control group; b: comparison with the induction group; c: comparison with the pentoxifylline group; **: P highly significant difference ($P < 0.01$); NS: no significant difference ($P > 0.05$)

Table 5: Histopathological Scoring System for Drugs Administered Topically Following Induction of Ischemia

Groups	Re-epithelialization	Granulation tissue	Inflammatory cells	Angiogenesis	Score
Control	Absence of epithelial proliferation with poor epidermal organization	Thin, immature, with normal components of skin appendages	1-2 cells/field	NO	5
Induction	Poor epidermal organization in $\geq 60\%$ of the tissue	Thin immature and inflammatory tissue in $\geq 60\%$ of the tissue	10-13 cells/field	1-2 vessels/site, Hemorrhage, congestion, with edema	1
Pentoxifylline Gel (1%)	epithelial remodelling in $\geq 80\%$	tissue organization in $\geq 80\%$	1-4 cells/field	vessels/site $\geq 7-8$	4
Cilnidipine Gel (1%)	Marked epithelial remodeling $\geq 80\%$	Basal layer regeneration $\geq 80\%$	1-4 cells/field	vessels/site $\geq 7-8$	4

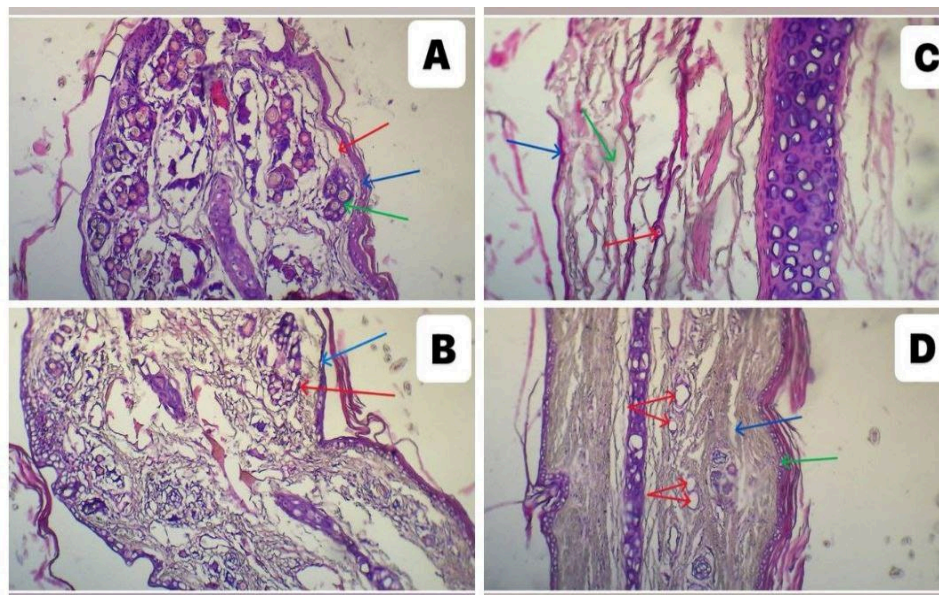


Figure 5: The skin sections of the experimental groups. A: The control group shows a normal appearance of the epidermis layer (blue arrow), normal dermal layer (red arrow), and hair follicle (green arrow). (H&E - 10X). B: the induction group shows incomplete epidermal organization (blue arrow) and moderate tissue remodeling (red arrow). (H&E - 10X). C: The pentoxifylline group shows epidermal re-epithelialization (blue arrow), Granulation tissue (green arrow), and vessels (red arrow). (H&E - 10X). D: the cilnidipine groups show complete re-epithelialization (blue arrow), complete tissue organization, and vessels (red arrow). (H&E - 10X).

Discussion

A chronic wound does not go through the regular healing steps in the correct order and on time. Managing chronic wounds is a significant challenge for healthcare professionals and individuals since the healing process is often prolonged or halted, resulting in extended patient suffering. Ischemia, mechanical pressure, reperfusion injury, and diabetes can turn an acute lesion into a chronic one in animals [16]. Many noteworthy findings suggest that rabbit wounds behave similarly to humans; the shared characteristics

include reduced scarring with age, collagen synthesis inhibitors and topical steroids, and increased scarring with delayed epithelialization [17].

Effect of Ischemic Induction on Inflammatory Mediators

The results of this study demonstrated a more significant elevation of inflammatory mediators, TNF- α and IL-6, in the tissue homogenate compared to the serum after the induction of ischemia. Following recruitment, leukocytes release various inflammatory mediators to reduce inflammation, including ROS, growth factors, and cytokines.

TNF- α and IL-6 all play essential roles in this process. Ischemic wounds showed significantly elevated levels of cytokines inflammatory chemokines at 7-14 days after an injury, there is an increase in inflammatory cells, resulting in increased monocyte recruitment that prevented granulation and epithelialization [18]. These monocytes undergo differentiation into inflammatory macrophages, which maintain the inflammatory response by the release of IL-6 and TNF- α .

Effect of Ischemic Induction on Matrix Metalloproteinase-9

In the current study, the findings indicate that the induction group exhibited elevated levels of MMP-9 in the tissue homogenate compared to the serum. This finding can be attributed to tissue hypoxia, which increases inflammatory cytokines such as IL-6 and TNF- α . These cytokines, in turn, stimulate the production of neutrophil gelatinase-associated lipocalin (NGAL). MMP-9 activity would interact with neutrophil gelatinase-associated lipocalin-NGAL, forming an (MMP-9/NGAL) complex during ischemia [19], resulting in elevated MMP-9 actions, which allowed the hemidesmosomes of basal epidermal keratinocytes to dissolve, hence preventing the keratinocytes from sticking together and allowing them to migrate [20]. When there is ischemic hypoxia, bacterial metabolic activity increases, producing high levels of MMPs, which has a negative impact on wound progression across the board because MMPs degrade the extracellular matrix (ECM) necessary for cell migration and cause the migration of immune cells, which initiates an inflammatory response.

Effect of Ischemic Induction on VEGF-A

VEGF-A levels in serum and tissue homogenate were significantly reduced after ischemia induction; this was attributed to the combined impact of ischemia and injury, which caused a brief delay in the production of VEGF-A. The results agreed with the previous study, which revealed that non-healing wounds exhibit elevated levels of inflammatory cytokines, including IL-1, IL-6, and TNF- α , increased levels of proteinases, and decreased growth factor activity as compared to fluids obtained from wounds in the acute healing phase [21].

Effect of Pentoxifylline Gel (1%) on Inflammatory Mediators

The regulation of inflammation plays an essential role in the process of wound healing since excessive activation of the inflammatory response may lead to a delay in this physiological process [22]. Pentoxifylline, a nonselective competitive PDE inhibitor (PDE-I), was applied topically after ischemia induction, and the levels of inflammatory mediators TNF- α , IL-6, and MMP-9 in serum and tissue homogenate were significantly reduced [23], inhibits leukocyte hyperactivity and lowers cytokine release by inhibiting neutrophil adherence and production of superoxide [24]. Serum and tissue homogenate levels of (TNF- α), (IL-6), and (MMP-9) in rabbits given topical pentoxifylline gel were significantly reduced in comparison with an induced non-treated ischemic group because of its action as a nonselective competitive PDE inhibitor (PDE-I), which increases intracellular cAMP, stimulates PKA, and decreases the production of TNF- α and IL-6 [25].

Effect of Pentoxifylline Gel (1%) on VEGF-A:

In this study, the topically applied pentoxifylline elevated VEGF-A levels in serum and tissue homogenate compared to the induction group due to enhancing RBC deformability and erythrocyte flexibility, allowing for easy vascularization and increasing tissue oxygenation and blood flow in the vessels. According to the results of the previous study, the administration of pentoxifylline showed an ability to enhance the process of fibrinolysis while reducing leukocyte hyperactivity by the reduction of neutrophil adhesion and superoxide release, improving microcirculation and oxygen delivery, especially in ischemic situations [26].

Effect of Cilnidipine Gel (1%) on Inflammatory Mediators

Serum and tissue homogenate levels of (TNF- α), (IL-6), and (MMP-9) in rabbits given topical cilnidipine gel showed no statistically significant differences compared to a group given topical pentoxifylline gel and significantly reduced in comparison with an induced non-treated ischemic group. Calcium channel blockers (CCBs) can reduce mitochondrial toxicity by preventing an increase in cytosolic Ca^{2+} [26], enhancing microcirculation [27], and suppressing the release of the mediator [28]. The possible mechanism behind the anti-inflammatory effects of Cilnidipine may include the inhibition of prostaglandin production. The synthesis of prostaglandins is dependent upon the influx of calcium ions. Hence, cilnidipine has anti-inflammatory properties by reducing inflammatory mediators [29]. Another study showed that the new calcium channel blocker cilnidipine relaxed human arteries via calcium channel antagonism and increased nitric oxide production via endothelial nitric oxide synthase enhancement and anti-oxidant effects [30, 31].

Effect of Cilnidipine Gel (1%) on VEGF-A

Serum levels of (VEGF-A) were significantly elevated among the cilnidipine group compared to the pentoxifylline group, and tissue homogenate levels of (VEGF-A) showed no statistically significant differences among the cilnidipine group compared to a group given topical pentoxifylline gel. Calcium channel blockers enhance endothelial superoxide dismutase activity by increasing vascular endothelial growth factor (VEGF) production by smooth muscle cells (SMCs) and enhancing the flow of blood to the injury site. CCBs increase angiogenesis by enhancing NO production by decreasing NADPH oxidase subunit expression and NADPH oxidase-mediated O₂ synthesis anti-oxidant effects [32]. In addition, it is known that CCBs have vasodilatory properties that increase blood flow to the injury site and stimulate growth factor synthesis.

Conclusion

Cilnidipine gel (1%) applied topically twice daily for 14 days resulted in a notable enhancement in the healing process of ischemic wounds in the rabbit ear model since it induced a significant reduction in inflammatory biomarkers, decrement in oxidative tissue damage, and increment in angiogenic activities accompanied this improvement.

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