Original Article

Evaluation of the Healing Effect of Nitrofurazone in Cutaneous Full-Thickness Wound in an Experimental Rat Model

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Abstract

Background: *Wound healing is a complex process involving coordinated cellular and molecular events to restore skin integrity. Full-thickness cutaneous wounds pose major challenges and demand effective treatment strategies. Nitrofurazone, a broad-spectrum antimicrobial agent, is used for treating various wounds, but its effectiveness in full-thickness wound healing remains insufficiently studied.*

Materials and Methods: *This study aimed to evaluate the healing effects of Nitrofurazone in a rat model of full-thickness cutaneous wounds. The experimental design included 14 rats, divided into control and Nitrofurazone-treated groups. Wound contraction, epithelialization period, histopathological analysis, and scar formation were assessed at multiple time points (1st, 4th, 7th, 10th, 14th, and 21st days). Statistical analyses included independent two-sample t-tests, paired t-tests, Mann-Whitney U tests, and Chi-square tests for different parameters.*

Results: *Significant differences in wound contraction were observed between the control and Nitrofurazone groups on the 4th and 7th days, with Nitrofurazone-treated rats showing improved wound closure. The epithelialization period was shorter in the Nitrofurazone group, but not significantly. Histopathological analysis revealed significant fibroplasia in the Nitrofurazone group. However, there were no significant differences in scar formation, angiogenesis, or inflammatory cell infiltration between the two groups.*

Conclusions: *Nitrofurazone demonstrated enhanced wound contraction and fibroplasia in the early stages of wound healing, suggesting its potential for improving wound healing dynamics. However, its effects on long-term outcomes, including scar formation, were not significant. Nitrofurazone could be a valuable antimicrobial agent in wound management, but its role in long-term wound remodeling and scar control needs further studies.*

Key words: *Full-thickness Wound, Fibroplasia, Nitrofurazone, Rat Model, Wound Healing.*

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Introduction

Wound healing is a complex and dynamic process that involves several cellular and molecular mechanisms responsible for restoring the skin's integrity and function after injury. Cutaneous wounds, particularly full-thickness wounds are a major challenge in clinical and experimental settings owing to the effect on quality of life, necessitating efficient management strategies for proper healing to occur.[1] Among the many topical agents for wound management, Nitrofurazone has emerged as a promising antibiotic with potential healing properties in the treatment of infected and non-infected cutaneous wounds.[2,3] It has been shown to prevent infection and promote healing by reducing the bacteria load and is thus an important tool in wound care.[4]

Nitrofurazone is a broad-spectrum antimicrobial agent frequently employed in the topical treatment of different kinds of wounds, such as burns and skin infections. It has been widely studied for its bacteriostatic and bactericidal activity, majorly against Gram-positive and Gram-negative bacteria.[5] However, besides its antimicrobial properties, Nitrofurazone has been proposed to contain secondary therapeutic properties that may contribute to wound healing, including the ability to improve tissue regeneration, decrease inflammation and promote

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collagen deposition.[6] Despite these promising indications, the extent to which it is effective in facilitating the healing of cutaneous wounds, especially for full-thickness wounds, is not well studied in scientific literature.

Full-thickness wounds, which penetrate the entire dermis and into the subcutaneous tissue, present special problems because the regeneration of both epithelial tissue and connective tissue must be considered. The healing of such wounds consists of a series of coordinated stages, including hemostasis, inflammation, and proliferation and remodeling.1 During these stages, the existence of infection and inflammation can critically prolong the process of healing, and the use of appropriate pharmacological agents is decisive in the management of these issues. Nitrofurazone, given its antimicrobial capacity, is frequently applied topically as a prophylactic and infection control measure to such wounds, which may help speed up the healing process. While several studies have shown antibacterial efficacy of Nitrofurazone in wound management, the exact role of Nitrofurazone in modulating the healing process, particularly in an experimental setting using the rat model, has not been well explored.[7]

This study is aimed to assess the healing effect of Nitrofurazone in cutaneous full-thickness wound in an experimental rat model. Specifically, this research will focus on the evaluation of the histopathological, biochemical and functional results of the presence of Nitrofurazone in the healing process, focusing on the regeneration of the tissue, collagen synthesis and wound contraction. The results of this exploration may give valuable information about the effectiveness of Nitrofurazone, as a therapeutic agent in the management of cutaneous wounds, and may lead to more effective wound care measures in the clinic. By investigating the dynamics of healing in a controlled experimental model, the aim of this research is to provide insight into the exploration of the healing potential of Nitrofurazone and how it may be applied clinically in the future in terms of wound healing.

Materials and Methods

The study was conducted at the Department of Pharmacology and Therapeutics, Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh, in collaboration with the Institute of Nutrition and Food Science, University of Dhaka, from January to December 2019. A full-thickness cutaneous wound model in rats was used to evaluate the therapeutic effect of 0.2% nitrofurazone cream by comparing wound contraction, epithelialization, collagen formation, and angiogenesis between treated and control groups. Fourteen healthy adult male Wistar rats (10-12 weeks, 150-200 g) were housed under standard laboratory conditions (12-hour light-dark cycle, 22 ± 2°C) with free access to food and water, following a one-week acclimatization period. Only healthy rats without prior injury or disease were included, while those with infection or abnormal healing were excluded. Ethical approval was obtained from the Ethical Review Committee of Sir Salimullah Medical College, and all procedures followed the guidelines of the National Institutes of Health for the care and use of laboratory animals. Under anesthesia with xylazine (5 mg/kg) and ketamine (80 mg/kg), a 2 × 2 cm full-thickness dorsal skin wound was created aseptically. Rats were divided into two groups: one treated with 0.2% nitrofurazone cream twice daily and the other receiving normal saline. Wound healing was assessed clinically by measuring wound area on days 1, 4, 7, 10, 14, and 21 using graph paper tracing, calculating percentage wound contraction, and recording epithelialization time. Scar characteristics were evaluated on day 21 based on color, contraction, and surface features. Histopathological analysis was performed on day 21 tissue samples fixed in formalin, processed, sectioned at 5 µm, and stained with hematoxylin and eosin. Microscopic evaluation using an Olympus BX205 microscope assessed fibroplasia, angiogenesis, epithelialization, and polymorphonuclear neutrophils, graded semi-quantitatively as absent (–), mild (+), moderate (++), or marked (+++). Data were analyzed using appropriate statistical tests: independent sample t-test for continuous variables (expressed as mean ± SD), Mann-Whitney U test for ordinal data, and Chi-square test for categorical variables. Observations were made by a blinded examiner across five random fields per specimen, and a p-value < 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics for Windows Version 22.0.

Results

Table I reports the wound surface area (mm^2) for both control and Nitrofurazone-treated groups at various days post-injury (1st, 4th, 7th, 10th, 14th, and 21st days). Significant differences were observed between the groups on the 4th and 7th days (Figure 1), showing that Nitrofurazone had an effect on wound contraction at those time points ($p < 0.05$). The rest of the days showed no significant differences.

Table I: Wound healing activity of Nitrofurazone by assessing wound surface area in rats.

Post-Injury Day	Control (n=07)	Nitrofurazone (n=07)	P-value (t-tests)
1st Day	352.14 ± 9.51	354.43 ± 7.98	0.643#
4th Day	326.71 ± 8.38	309.29 ± 5.88	0.0007*
7th Day	236.14 ± 11.94	219.28 ±13.30	0.028*
10th Day	112.86 ± 16.79	100.43 ± 12.96	0.147#
14th Day	21.71 ± 8.38	24.29 ± 12.46	0.657#
21st Day	2.86 ± 2.04	1.43 ± 0.98	0.120#

Values are expressed as Mean ± SD wound surface area (mm2) on different days post-injury. Independent two-sample t-tests were done. * Denotes statistically significant result. # refers to statistically non-significant result.

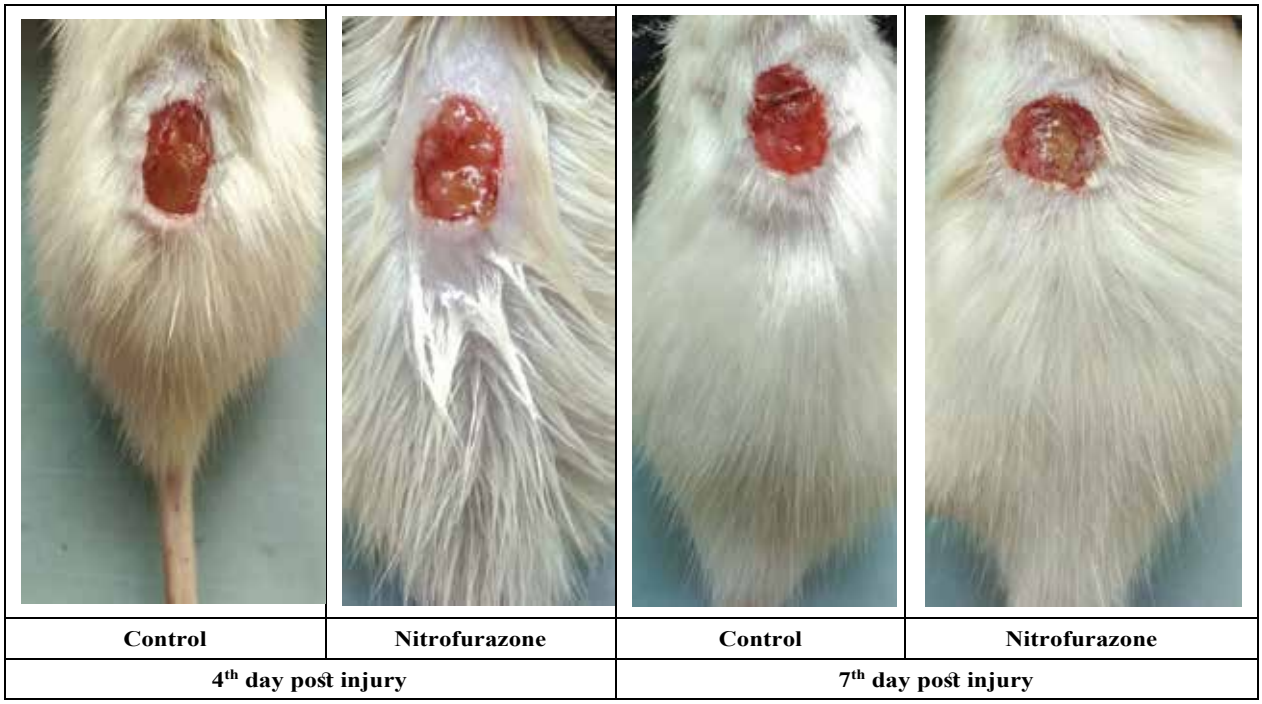


Figure 1: Photographs of rat cutaneous full-thickness wound at 4th, and 7th day post injury.

Table II compares the percentage of wound contraction between the control and nitrofurazone groups in rats on different post-injury days. Nitrofurazone-treated rats showed slightly better wound contraction percentages, particularly on the 4th, and 7th ($p<0.05$) (Figure 1).

Table II: Comparison of wound contraction (%) between control and Nitrofurazone group in rats at different post-injury days

Post-Injury Day	Control (n=07)	Nitrofurazone (n=07)	P-value (t-tests)
1st Day	11.96 ± 2.38	11.43 ± 2.01	0.657 #
4th Day	18.32 ± 2.10	23.32 ± 1.02	0.0001*
7th Day	40.96 ± 2.98	45.18 ± 3.33	0.028 *
10th Day	71.79 ± 4.20	74.89 ± 3.24	0.148 #
14th Day	94.57 ± 2.10	93.93 ± 3.11	0.659 #
21st Day	99.29 ± 0.51	99.64 ± 0.24	0.126 #

Values are presented as Mean ± Standard Deviation (SD). P-values calculated using independent sample t-test. *Denotes statistically significant result. # refers to statistically non-significant result.

Table III demonstrates the epithelialization period of the control and nitrofurazone groups on wound healing in rats. The epithelialization period (the number of days it takes for the wound to heal at the surface) was compared between the control and nitrofurazone groups. Nitrofurazone treatment resulted in a shorter epithelialization period (15.58 days) compared to the control (17.57 days), but this difference was not statistically significant ($p = 0.159$).

Table III: Epithelialization period of control and Nitrofurazone group on wound healing in rats.

Group	Epithelialization period (Days)	*p*-value
Control (n=07)	17.57 ± 2.64	0.159#
Nitrofurazone (n=07)	15.58 ± 2.30	

Values are expressed as Mean ± SD epithelialization period (days) in treated and control groups. An Independent Two-Sample t-test was done. # refers to statistically non-significant result.

Table IV illustrates the histopathological survey in the control and treatment groups on the 21st day post-injury. This table compares histopathological features like fibroplasia, polymorphonuclear neutrophils, angiogenesis, and epithelialization between the control and nitrofurazone groups on day 21. Fibroplasia was significantly higher in the nitrofurazone group. However, other features like neutrophils, angiogenesis, and epithelialization showed no significant differences (Figure 2).

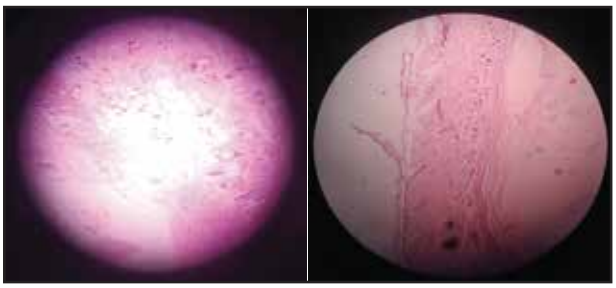
Table IV: Histopathological survey in the control and treatment groups on the 21st day post-injury (n=14)

Circumstances wound healing progresses	Control	Nitrofurazone	P value
Fibroplasia	++	+++	0.00041*
Polymorphonuclear neutrophils	–	–	1.0#
Angiogenesis	+	+	1.0#
Epithelialization	+	+	1.0#

Statistical test: Mann–Whitney U test was used to compare the groups.

Significance level: $P < 0.05$ considered statistically significant.

* indicates statistically significant difference ($P < 0.05$); # indicates non-significant difference ($P \geq 0.05$).



Control group Nitrofurazone treated group

Figure 2: Photomicrograph of healed cutaneous wound of control group shows thick collagen fibers along with numerous fibroblasts, the absence of neutrophils, and the existence of few blood vessels. The Nitrofurazone-treated group reveals abundant thick collagen fibers along with few fibroblasts, absence of neutrophils, existence of 0 -2 blood vessels (10X, H&E).

Table V shows examination of scar color in control and nitrofurazone. The scar color was assessed on days 7, 14, and 21 post-injury, with categories including normal, pink, red, purple, and dark purple. No significant differences were found between the control and nitrofurazone groups across these days ($p > 0.05$).

Table V: Examination of scar color in control and Nitrofurazone (n = 21)

Status of scar color & experimental groups	Normal	Normal center with scar ring	Pink	Red	Purple	Dark Purple	*P-value*
7th day post injury							
Control	0	0	0	4	3	0	0.126 #
Nitrofurazone	0	0	3	3	1	0	
14th day post injury							
Control	0	3	4	0	0	0	0.554 #
Nitrofurazone	0	1	6	0	0	0	
21st day post injury							
Control	1	6	0	0	0	0	1.0 #
Nitrofurazone	0	7	0	0	0	0	

Chi-Square test was performed to evaluate level of significance. # refers to non-significant.

Table VI shows the result of the examination of scar border contraction between the control and nitrofurazone-treated groups. There were no significant differences in the contraction of scar borders between the control and nitrofurazone groups at any of the time points.

Table VI: Examination of scar border contraction in control and Nitrofurazone (n = 14)

Status of scar border contraction & experimental groups	Normal, smooth, oval	Some border irregularity	Most of border contracted	Severely contracted edge	P Value
7th day post injury					
Control	0	7	0	0	1.0 #
Nitrofurazone	0	7	0	0	
14th day post injury					
Control	3	4	0	0	0.577 #
Nitrofurazone	2	5	0	0	
21st day post injury					
Control	7	0	0	0	1.0 #
Nitrofurazone	7	0	0	0	

Chi-square test was performed to evaluate the level of significance. # refers to not significant.

Table VII shows the comparison of the final appearance of the scar in the control and nitrofurazone groups. The final appearance of scar (excellent, minor scar, moderate scar, severe scar) was evaluated. No significant differences were observed between the two groups regarding scar appearance (p = 0.84246).

Table VII: Examination of final appearance of scar in control and Nitrofurazone

Final Appearance	Control (n=07)	Nitrofurazone (n=07)	*P* value
Excellent	3	2	0.84246#
Minor scar	3	4	
Moderate scar	1	1	
Severe scar	0	0	

refers to statistically non-significant result.

Discussion

The purpose of this study was to determine the healing effect of Nitrofurazone in full thickness cutaneous wounds in experimental rat model based on wound contraction, epithelialization, histopathological changes, and scar formation. The results obtained suggest that Nitrofurazone had a significant effect on wound contraction on some days after causing the injury, namely on the 4th and 7th day but no long-term significant difference was found on the 21st day when compared with the control group. The epithelialization time was also slightly reduced in the Nitrofurazone treated group, but the difference was not statistically significant. Histopathological findings showed that fibroplasia was significantly increased in Nitrofurazone-treated group, which indicates an increase in collagen formation, which is important in wound healing. These findings favor the use of Nitrofurazone as a potential promoter of wound healing, although the overall effect on scarification and final appearance was not significant.

Wound healing is a multi-phase process which includes hemostasis, inflammation, proliferation, and remodeling where each phase is involved in wound regeneration and restoration of skin integrity. However, infection or chronic inflammation can severely impair this process and the use of antimicrobial agents is therefore essential for successful healing.[1,3] Nitrofurazone is an antimicrobial agent that has long been used in clinical practice for the treatment of many different kinds of wounds, especially those associated with infection. Its potential therapeutic effects are not limited to infection control but also related to tissue regeneration, inflammation and collagen deposition.[5,6]

In the present study, the wound contraction of the Nitrofurazone-treated rats was improved in comparison with the control group at the 4th and 7th days post-injury. This result is in accordance with previous studies that have reported improved wound healing with Nitrofurazone in infected and

non-infected wounds.[2,8] Although the differences were only statistically significant in the earlier stages of healing, the direction of these differences, towards faster contraction in the Nitrofurazone group, is consistent with the antimicrobial properties of nitrofurazone, which may decrease bacterial load and aid in faster resolution of the inflammatory process and therefore lead to faster wound closure.[9]

The epithelialization period was less in the Nitrofurazone treated group but not significantly. This suggests that while Nitrofurazone may have some impact on accelerating epithelial closure, its impact on epithelialization may be secondary to its antimicrobial effect, rather than having a direct impact on stimulating cellular migration or proliferation.[1] Similar results have been documented in previous studies in which Nitrofurazone has been shown to decrease the time for epithelialization of various wound models, though statistical significance was not always reached.[7,10]

Histopathological examination showed increased fibroplasia in the Nitrofurazone group with an abundance of thick collagen fibers, hence, better tissue regeneration and remodeling. This result is consistent with previous reports that Nitrofurazone is capable of inducing collagen deposition in the wound bed.[5,6] Collagen is important for the structural strength of the wound, as it gives it tensile strength and aids tissue regeneration.1 The enhanced fibroplasia in this study indicates that Nitrofurazone might affect the proliferation of fibroblasts and synthesis of extracellular matrix proteins and thus may shorten the maturation stage of wound healing.

However, even though there were positive effects on wound contraction and fibroplasia, no significant differences were found between the Nitrofurazone and control groups in the final appearance of scars, which are consistent with other studies showing that Nitrofurazone has no real effect on scar formation.[2,4] No difference in scar color and border contraction between groups at different post-injury time intervals was found, thus suggesting that although Nitrofurazone may enhance early healing processes, it has little effect on long-term cosmetic outcome like scar maturation.

Furthermore, the lack of significant differences in inflammatory cell infiltration, angiogenesis and epithelialization corroborate the idea that the effect of Nitrofurazone is mainly antimicrobial and not a direct influence on these other aspects of the healing process. While there are studies indicating that Nitrofurazone can have an effect on angiogenesis and inflammation, this study did not see clear evidence of those effects in the rat model employed.[6]

Nitrofurazone has shown important effects in the early phase of wound healing on contraction and fibroplasia but has a limited overall effect on wound healing, particularly when it comes to epithelialization and scar formation. These findings add to the literature regarding the use of Nitrofurazone in wound care and indicate that although it may promote early closure of wounds, its impact on long-term wound remodeling and scar formation is less likely compared to other modalities.

In the future, the integration of Nitrofurazone with other therapeutic agents should be investigated to examine the possibility of synergistic effects on wound healing. In addition, clinical trials to determine the long-term effect of Nitrofurazone on scar formation and tissue regeneration in human beings are required to fully understand its therapeutic potential.

Conclusion

This study was designed to assess the effect of Nitrofurazone on full thickness cutaneous wound healing in an experimental rat model. The findings show that Nitrofurazone greatly enhanced wound contraction on the 4th and 7th day after injury compared with the control group, implying possible effectiveness of Nitrofurazone on early wound healing. However, there was no significant difference in epithelialization time, the appearance of the scar or final wound contraction at day 21, suggesting that its effect on long-term healing outcomes was modest.

Histopathological study showed significantly higher fibroplasia in Nitrofurazone group which indicates higher collagen synthesis and tissue regeneration. However, there were no significant differences in other histological parameters including inflammatory cell infiltration, angiogenesis and epithelialization. These results provide support for the hypothesis that Nitrofurazone may have its main effect in early wound healing by affecting the antimicrobial and collagen promoting properties, rather than acting directly on the other phases of the wound healing process. Nitrofurazone is a promising topical preparation for wound contraction and fibroplasia, but has limited effects on long-term outcome including scar formation. Future research can investigate combination therapies with other wound healing agents to see if the effects of Nitrofurazone can be boosted for overall healing and scar management.

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