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# Effect of Topical Application of N-Acetyl Cysteine and Vascular Endothelial Growth Factor on Cutaneous Wound Healing

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## ABSTRACT

Safety and effectiveness of topical N-acetyl cysteine (NAC) and vascular endothelial growth factor (VEGF) in the promotion of wound healing because of their in vivo anti-inflammatory and antioxidative effects. **Objectives:** To assess the effectiveness of topical NAC and VEGF, alone and combined, in accelerating burn wound healing. **Methods:** This randomized controlled laboratory pilot study used aseptic methods, two square skin burns were inflicted using HCl laterally on nine anaesthetized Sprague Dawley rats. The treatments, namely vaseline, vaseline and 3% NAC, VEGF (intradermal), and 3% NAC + VEGF, were topically administered separately. The wound healing process was determined by erythema, exudate, development of crust, swelling, as well as ulceration. **Results:** The 3% NAC + VEGF combination accelerated wound healing, visible through better and faster epithelization, while no significant difference in wound healing was observed with vaseline treatment alone as indicated by the histopathological results. **Conclusions:** In conclusion, the study highlighted the potential effectiveness of NAC and VEGF to overcome conditions associated with slow healing in chronic burns and to prevent secondary bacterial as well as fungal infections.

## INTRODUCTION

Thermal wound healing is a complex yet essential process in both human beings and animal beings whereby there are various overlapping stages [1]. The initial step, i.e. the inflammatory one, takes several days, and consists of hemostasia, chemotaxis, and increased vascular absorbency. This restricts further wound damage, eliminates cellular debris and bacteria, stimulates cell migration and thus, leads to wound healing. The second stage, which is called proliferative, takes several weeks and is marked by the formation of granulation tissue, re-epithelialization and neoplasia. Lastly, in the remodeling stage, the wound is the most potent as it continues to develop [2]. This cascade necessitates many growth factors such as transforming growth factor- beta (TGF- -1 ) platelet activating factor (PAF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) that seem to be the necessities to commence and enhance healing [3]. In most types of wounds including those that have been as a result of burn, vascular endothelial growth factor (VEGF) is produced by many types of cells which include the macrophages, endothelial cells and monocytes to hasten the healing process. VEGF is also reported to be expressed in the monocytes in the exudation phase of burns [4]. Burns are accompanied by complex changes to the pathophysiological environment of damaged cells leading to the detrimental impact of various organ systems, including the changed lipid peroxidation [5]. Moreover, such harmful effects are conditional on the presence of free radicals in various inflammatory cells such as macrophages, neutrophils, fibroblasts, and endothelial

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cells which are very reactive and therefore produce lipid peroxides. To combat cellular damage against these species, antioxidants are released thus maintaining cellular redox balance [6, 7]. Nuclear GSH is a major regulator which is involved in the cell proliferation regulation which is an important part of wound healing [8]. N-acetyl cysteine (NAC) has been identified to promote cellular production and release of GSH in case of redox imbalance, thus protecting the cells [9]. Due to this, several reports have identified the role of NAC in the treatment of respiratory diseases, as an antioxidant and anti-inflammatory substance that protects cells from damage caused by injuries including thermal injuries as well as in treatment of diabetes, cancers, cardiovascular conditions, metal poisoning, and liver toxicity from paracetamol. NAC has also shown to control the activity of several proteins, extends cell survival by delaying apoptosis, cartilage erosion, fibrosis, invasion, endothelial dysfunction, and acetaminophen detoxification, and hence the need for transplantation [10-12]. For treatment purposes, NAC is given orally, intravenously, and recently been suggested to be used as a topical treatment to burn wounds. However, the underlying mechanisms accelerating wound healing in response to tropical application of NAC remain yet to be elucidated. Furthermore, no commercially available NAC product has elucidated the role of endothelial growth factors as well as oxidative stress quenchers.

This study aimed to determine the efficacy as well as safety of the topical treatment of NAC and VEGF based on their in vivo antimicrobial, anti-inflammatory, antioxidative activity to accelerate wound healing in burn wounds.

## METHODS

This randomized controlled laboratory pilot study was conducted to evaluate the feasibility and preliminary efficacy of combined NAC and VEGF treatment on burn wound healing using a within-subject design. The study was conducted from February 2024 to August 2024 at Institute of Molecular Biology and Biotechnology, University Lahore. Ethical consent was obtained from University of Lahore and study was conducted according to institutional and NIH guidelines. Nine pathogen-free male Sprague Dawley rats (200–300 g) were used, each receiving two standardized burn wounds (1.2 × 1.2 cm) on opposite flanks, resulting in 18 wounds in total. Wounds were randomly assigned to four treatment groups: Vaseline (control), VEGF alone, Vaseline + 3% NAC, and 3% NAC + VEGF, with 4–5 wounds per group. Randomization was performed using a computer-generated sequence, and investigators involved in wound measurement, histology, ELISA, and PCR analyses were blinded to treatment allocation. A priori sample size calculation ($\alpha = 0.05$, power =0.8, effect size d = 0.8) indicated that approximately 25 wounds per group would be needed for sufficient power; hence, this study served as a pilot to establish feasibility and estimate effect sizes for future trials. Rats were housed under standard conditions (22–25°C, 12-hour light/dark cycle) with ad libitum food and water. Anesthesia was administered using ketamine (100 mg/mL) and xylazine (20 mg/mL). Burns were induced by applying HCl-soaked filter paper for 10 seconds, followed by sterilization with alcohol swabs and application of paraffin gauze and bandages. Treatments were applied daily; topical agents (Vaseline or NAC) were spread directly over the wound, and VEGF was injected intradermally. Postoperative monitoring ensured animal welfare, with humane endpoints defined for pain, infection, or impaired mobility. Wound contraction was assessed on days 1, 7, 10, and 14 using graph paper, and results were expressed as percentages. Skin tissues were collected, frozen in liquid nitrogen, and processed for biochemical, histological, and molecular analyses. To extract proteins, 1 mL of RIPA buffer was put in crushed tissue and centrifuged at an approximate of 14,000 x g. In the case of RNA isolation, TRIzol was applied and then chloroform extraction, isopropanol precipitation and ethanol washing were done. RNA that was extracted was kept at -20 o C and reverse transcended into cDNA. IGF-1, VEGF, PCNA, p53 and 8-actin (housekeeping gene) primers were used in the PCR. The amplification conditions were 95 o C 45 s, 60 o C 45 s, and 72 o C 45 s. Primary gene expression was determined through qRT-PCR with the use of SYBR Green and 2- -Ct relative to methodology analysis. To determine the tissue regeneration and epidermal restoration, histological analysis of the H&E-stained sections of the wounds (10x magnification) was conducted. The content of hydroxyproline was taken to determine the amount of collagen being produced after the 24 hours of acid hydrolysis at 50 C. VEGF, IGF and CD90 ELISA assays were conducted to assess the expression of proteins using the standard protocols. The effects of oxidative stress were determined through GSH and catalase tests through the use of absorbance at 412 nm and 530 nm, respectively. Graphpad Prism was used to perform the data analysis in v9.5.1. Normality was tested using the Shapiro-Wilk test ($p > 0.05$), and homogeneity of variances was confirmed by the Brown-Forsythe test. Repeated measures were analyzed using mixed-effects models with the animal as a random factor, while one-way or two-way ANOVA followed by Tukey's post-hoc test was applied for single-time-point comparisons. All quantitative assays were performed in duplicate or triplicate, and results were expressed as mean ± SD, with statistical significance set at $p \leq 0.05$.

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# RESULTS

Day 14 histological examination showed that the three experimental groups were different in their distinct ways. The control group (VAS) experienced cytological imbalances, epidermis disruption and the development of edema. Scar formation was observed in the VAS+NAC group, which means incomplete healing. HEAL VEGF+NAC showed a higher level of healing as there were certain signs of epidermis restitution and skin adnexa, which is indicative of synergistic wound repair stimulation by NAC and VEGF (Figure 1).

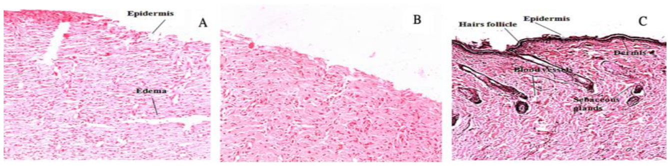


**Figure 1:** Histological Assessment Of Skin Tissue (A) Control Group (Vas), (B) Vas+Nac Group; C, Vas+Vegf Group

Measurement of wound contraction was done at day 1, day 3, day 7, day 10, and day 14. VEGF+NAC-treated rats exhibited a much faster wound healing than the VAS+NAC and control groups. In particular, the rate of wound contraction improved in the V EGF+NAC group by 18.44% on the day 3 to 41.28% on day 7 and 95.59% on the day 14. NAC was inducing moderate contraction (17.58, 35.56, and 88.70 on days 3, 7, and 14), compared to the lowest contraction of 9.38, 30.43 and 82.42 in the VAS control group (Figure 2).

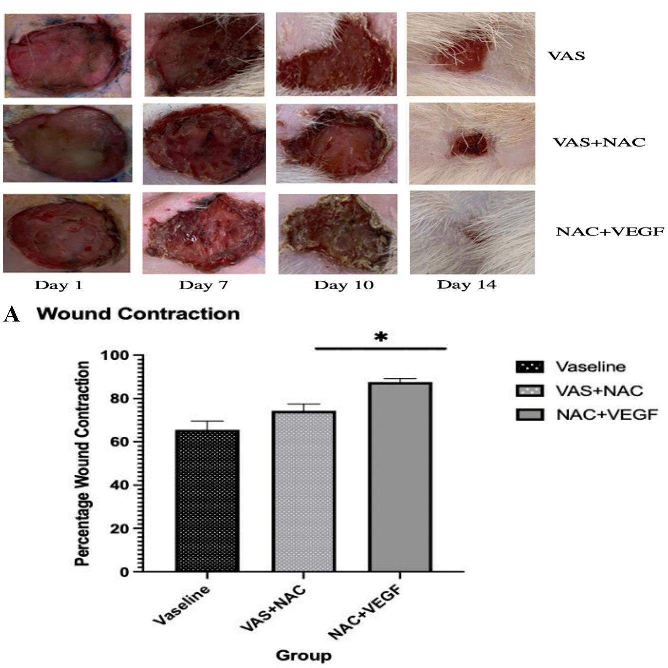


**Figure 2:** Effect of NAC And VEGF on Wound Healing (A) Wound Contraction Area in Vas, Vas + NAC, And NAC + VEGF Group. Wound Appearance Following 14 Days of Treatment with NAC + VEGF. (B) Percentage Wound Contraction. *p=0.004

The collagen synthesis markers (Hydroxyproline content) showed that in VEGF+NAC (1.14 ± 0.001) were significantly more than VAS+NAC (1.225 ± 0.151) and control (0.56 ± 0.028). High levels of hydroxyproline mean that there is an increased activity in collagen deposition and wound healing (Figure 3).

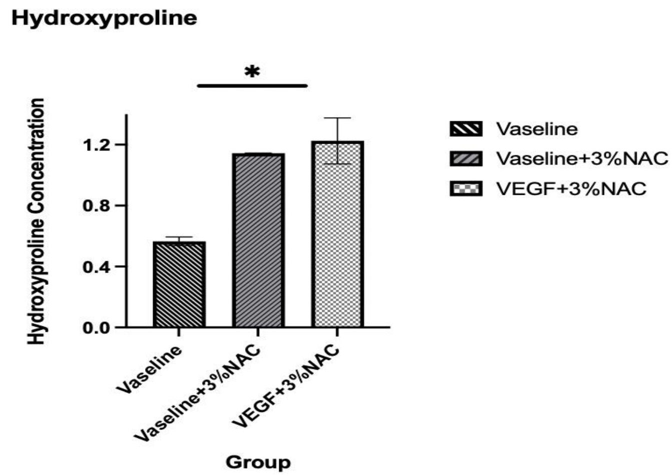


**Figure 3:** Concentration of Hydroxyproline in VAS, VAS+NAC, and NAC+VEGF Treated Groups. *p=0.002

The levels of IGF-1 and CD90 expressed by the VEGF+NAC group were found to be elevated compared to VAS+NAC and control groups using ELISA. IGF-1 is known to stimulate the proliferation, collagen synthesis, and epithelialization of the cell whereas CD90 is known to stimulate fibrosis, tissue building, and inflammation which facilitated the enhanced healing of the VEGF+NAC group (Figure 4).

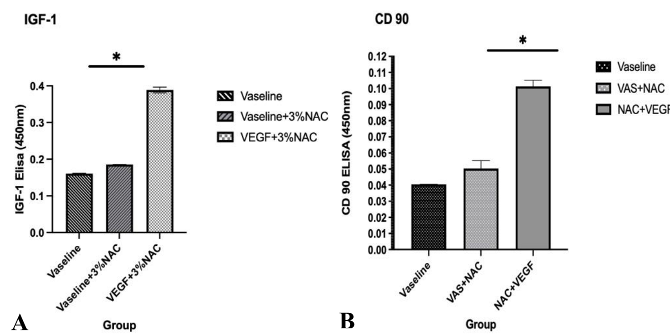


**Figure 4:** Effect of NAC and VEGF on IGF-1 and CD90. (A) IGF-1. *p=0.001 (B) CD90. *p=0.001. Error Bars Represent Standard Error of Mean (SEM)

Topical NAC treatment enhanced the activity of antioxidant enzymes such as catalases and glutathione (GSH) than the control group. The highest levels were indicated in the VEGF+NAC therapy, which reduces the effects of oxidative stress and promotes the healing of the wound (Figure 5).

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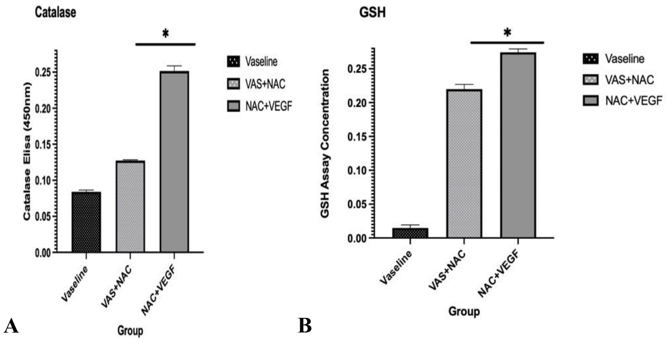


**Figure 5:** Effect of NAC and VEGF on Oxidative Stress Markers. (A) Catalase. *p=0.001 (B) GSH. *p=0.001. Error Bars Represent Standard Error of Mean (SEM).

IGF-1, 827 b-actin, PCNA, SD, HIF-1, c-kit, CXCR4, and P53 were amplified by PCR, and their expression demonstrated increased expression of VEGF +NAC wounds relative to VAS +NAC and controls (Figure 6). High affinity to epithelial growth factor, integrin, and cytokine cDNA primers suggests that VEGF+NAC promotes transcriptional activity of wound healing importance (Figure 6).

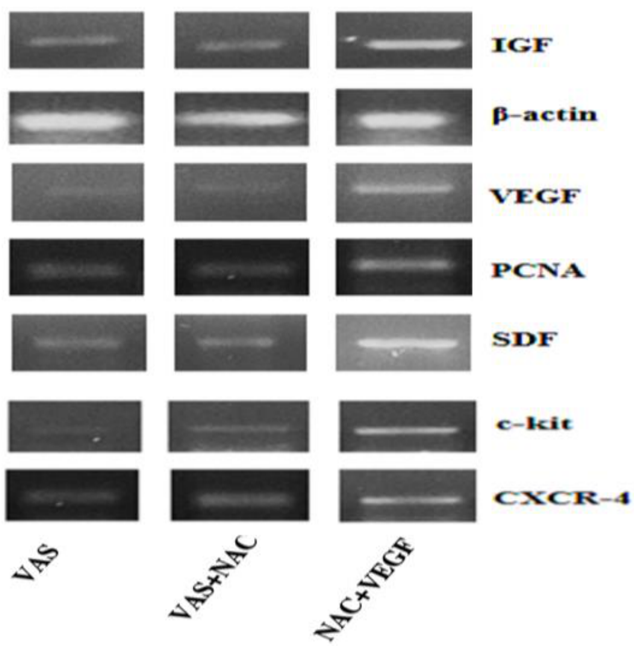


**Figure 6:** Effect of NAC+VEGF on Gene-Expression Analysis of Rats Imposed with Burn Injury

## DISCUSSION

The findings indicate that the NAC and VEGF treatment regimen has a significant effect on accelerating the healing of burn wounds and is better than NAC treatment or the control group. Histological and wound contraction evidence indicate that there are synergistic effects, where VEGF+NAC enhances re-epithelialization and scar formation and skin adnexa restoration. The level of hydroxyproline was also found to be increased, which is in line with the prior research that had indicated that the proline/hydroxyproline supplementation positively influences wound healing rate [13-15]. Another reason why the growth factors and cellular receptors are important in the tissue repair process is verified by enhanced IGF-1 and CD90 levels in VEGF+NAC-treated wounds that facilitate tissue repair, proliferation, and fibrosis modulation [16-18]. Topical NAC was able to increase antioxidant defenses through catalases and GSH and reversed oxidative stress and cell repair. VEGF probably adds to this effect by enhancing angiogenesis to provide an environment that facilitates wound healing [19-21]. PCR outcomes reveal an up-regulation of several genes that are essential in cell proliferation, migration and repair such as PCNA, HIF-1alpha and c-kit, which prove the transcriptional up-regulation behind the observed physiologic healing response. Altogether, the synergistic action of VEGF+NAC enhances the wound healing of burns with co-regulated collagen deposition, growth factors signaling, antioxidative stress, and tissue regeneration-related gene expression.

## CONCLUSIONS

NAC has widely been administered orally and topically for increases wound healing due to its strong antioxidant properties, and the role of VEGF in angiogenesis and therefore wound healing is also well established. However, using a combination of NAC and VEGF to subsequently increase burn wound healing in rat model has not been established yet. The present study showed that such a combination of NAC and VEGF increases cell proliferation, angiogenesis, CD90 as well as IGF-1 mediated wound healing. Our results show that NAC and VEGF can be used topically to potentially increase wound healing and can be used as a promising model for burn wound healing in humans.

## Authors Contribution

Conceptualization: FA
Methodology: OS
Formal analysis: FA
Writing review and editing: FA, NW, MSN

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

All the authors declare no conflict of interest.

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