

ORIGINAL ARTICLE

ANGIOGENESIS ACCELERATION IN WOUND HEALING:
ZINC OXIDE VERSUS BETEL LEAF EXTRACTMuhammad Ali Zubair, Suhail Marfani*, Javed Mahmood**, Farheen Hameed,
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Background: Wound healing is a complex physiological process involving haemostasis, inflammation, proliferation, and remodelling. Chronic wounds pose challenges due to delayed healing. This study compares the effects of topical zinc oxide (ZnO) and betel leaf extract on angiogenesis in wound repair. **Methods:** This study was carried out over 6 months from Oct 2023 to Mar 2024, at Shahida Islam Medical Complex, Lodhran. Thirty-six albino rats were divided into three groups of 12 each. A dorsal wound was created, and treatments were applied to the wound. Group A was control, and saline was applied to the wound, Group B was administered 20% ZnO on the wound, and Group C received betel leaf extract as wound dressing. Wound margin tissue was extracted, fixed, and histopathological slides were prepared. Masson trichrome stain was used to count vessels at 400× magnification under microscope. Vessel counts were analyzed via microscopy on days 3, 7, and 14. Data was analysed using one-way ANOVA. **Results:** On day 3, vessel counts were 4.3 for group A, 6.5 for group B, and 4.2 for group C. By day 7, counts increased to 5.8 for A, 11.54 for B, and 9.52 for C, and on day 14, they were 5.5 for A, 7.54 for B, and 6.57 for C. ZnO showed a significant increase in angiogenesis compared to control ($p < 0.01$) and betel leaf extract ($p < 0.05$). **Conclusion:** Zinc oxide accelerated wound healing more effectively than betel leaf extract.

Keywords: Angiogenesis, Wound repair, Zinc oxide, Betel leaves

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INTRODUCTION

Wounds especially the chronic wounds have a negative impact on the quality of life of nearly 2.5% of American population, a significant economic impact on the healthcare.¹ Chronic wounds as well as burn injuries have been increasing, and have led to an increasing cost for the national health systems of many countries. Development of new, improved, and cost-effective therapy is a huge area of academic research. It is also critical for long-term sustainability of public healthcare system.² Many materials have been used for improving wound healing and all of them have their pros and cons. Recently, nano-zinc oxide has been used in wound healing applications due to its promising microbicidal and anti-inflammatory properties as it provides a rich source of zinc ions.³ Topical zinc oxide is being used and heavily studied upon since years, and is stated that it is highly underappreciated even though clinical evidence suggests its facilitation in epithelialization, anti-infective action, and auto debridement.⁴ Established perfusion at any stage of wound repair is required for faster process, either in acute or chronic cases.⁵ Vascular system promptly begins the healing process by triggering the coagulation cascade and initiating vasoconstriction.⁶ As the cutaneous wound occurs in the body, the different inter and intra cellular pathways play role to restore the cellular and tissue integrity.^{7,8}

Zinc oxide (ZnO) is a trace element that plays a physiological function in healing. It is an essential cofactor for multiple metalloenzymes, needed for cellular repair, tissue regeneration and immune booster.⁹ Molecular and cellular function of zinc incorporates in modulating the repair process. Zinc dependent proteins take part in transcription regulation, metabolic process, and antioxidant process. Free zinc ions working as second messengers like calcium, are capable to target and stimulate many signal transduction pathways.¹⁰

Betel leaf is one of the effective leaves that can accelerate many cellular and biochemical properties that can accelerate the healing process. It is a harmless leaf composed of eugenol, anethole, chavicol and caryophyllene.¹¹ The leaves possess potent antimicrobial properties and are effective in both acute and chronic wound conditions.¹² Betel leaves have anti-inflammatory action and can trigger angiogenesis to stimulate the physiological and pathological function as well. By having impressive modulator effect on immune system, the healing process can be accelerated in any type of wound.¹³ Betel leaves contain saponins and flavonoids that are composed of antioxidant properties, and facilitate angiogenesis which results in acceleration of epithelialization process.¹⁴

The aim of this study was to compare the two different healing agents, topical application of zinc oxide and betel leaf extract.

METHODOLOGY

A quasi-experimental study was done at Shahida Islam Medical Complex, Lodhran, Pakistan for 6 month from Oct 2023 to Mar 2024 after the ethical approval from the Institutional Ethical Review Committee vide No. SIMC/HR/7727/23 dated 1st Sep 2023. Randomly selected 36 male albino rats weighing 150–200 gm were taken from animal house of the Institute. Animals were divided into 3 groups of 12 animals each and further sub-divided into 4 units according to day of sampling. The animals were kept in separate cages according to the grouping with maintenance of 12/12 hour light and dark cycle. Group A (Control group) was treated with topical application of normal saline once a day over the site of the wound. Group B was given 20% of ZnO topically once daily. Group C received the green betel leaf extract.

The extract was made with about 25–30 fresh leaves bought from the local market, dried in incubator at 40 °C for 24 hours, ground to powder form, and stored in glass container till use. The ground leaves were placed in distilled water for 24 hours and then filtered using Whatmann No. 4 filter paper. The filtered extract was placed into glass bottles.¹⁵ The dose of the betel leave extract was soaked in wound using gauze twice a day, as much as 2 drops.

The dorsal surface of the animals was shaved and cleaned with normal saline, and wounds of 2.5×2.5 Cm were prepared with a blade. The process of healing was observed by the process of angiogenesis. The samples were taken from each group on different days. The tissue was extracted from the margin of the wound, and kept in formalin. The histopathological slides were prepared to analyse the numbers of vessels and stained with Masson trichrome stain was used to calculate the numbers of vessels in specific field area under the light microscope at 400× magnification. The mean number of vessels was compared between the groups applying one-way ANOVA and $p \leq 0.05$ was considered as statistically significant.

RESULTS

The number of vessels (Mean±SD) on day 3, day 7 and day 14 is shown in Figure-1. Statistical significance (p -values) of differences in mean blood vessel counts among different groups is shown in Table-1.

Figure-2 shows histomorphological appearance of process of angiogenesis number of vessels among different groups at 400× magnification. Group A (control group) showed decreased number of vessels. Group B showed significant increase in number of vessels as compared to Group C. The Group C treated with betel leaf extract also showed increase number of vessels as compared to control group but to a lesser number as compared to Group B.

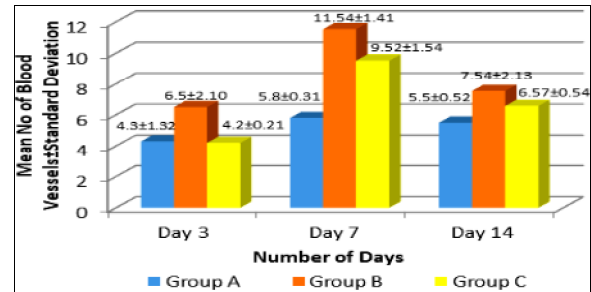


Figure-1: Number of blood vessels in different therapeutic groups on different days of sampling

Table-1: Comparison of blood vessel counts among different groups (p -values, One-way ANOVA)

Groups	Day 3	Day 7	Day 14
B versus A	0.001	0.001	0.002
B versus C	0.001	0.001	0.004
A versus C	0.08	0.001	0.04

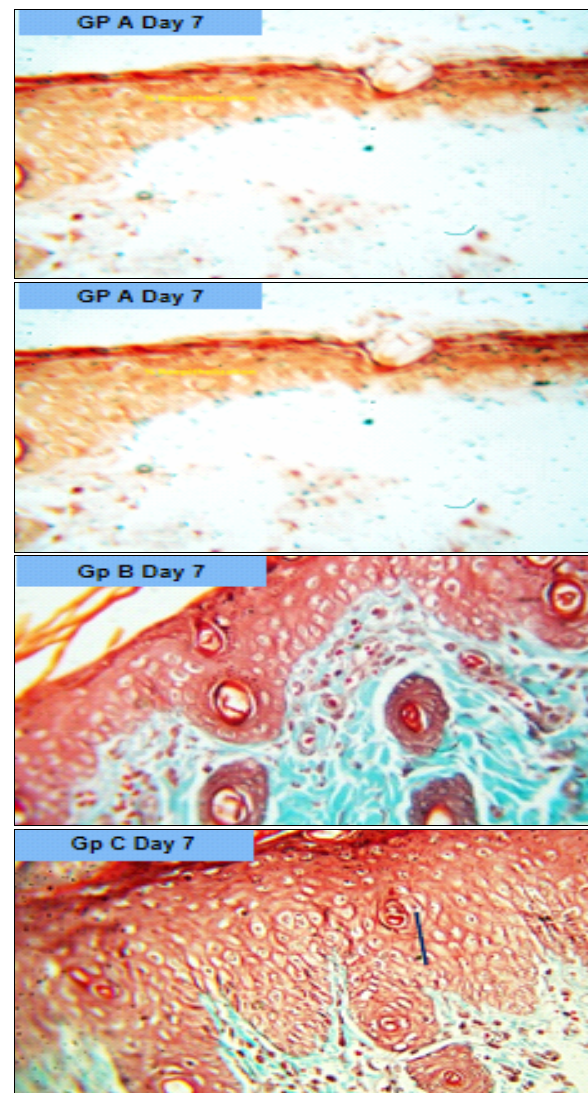


Figure-2: Photomicrograph showing process of angiogenesis among different therapeutic groups

DISCUSSION

We used betel leaf extract and compared it with topical zinc oxide to see their effects on the number of blood vessels formed. The mean number of vessels formed through angiogenesis was greater for zinc oxide topical application as compared to betel leaf extract on all days the sample was taken. A similar study reported comparable results, with an alpha score of 0.002, indicating a significant difference in injury healing when using red betel leaf extract in two doses per day as compared to a single dose. The wound healed in 6 days as compared to 8 days when used single dose per day, and 10 days when used povidone iodine alone.¹⁴ Agren *et al*¹⁶ compared the topical application of zinc oxide in two human wound models. They monitored wound healing by considering scab formation as criteria for complete healing. They found ZnO more effective in healing of wounds on different days. This supports the current study findings.

Lien *et al*¹³ observed that the betel leaf extract showed better percentage in healing of burns on 2nd, 4th, and 6th postoperative days of burns; and on excisional wounds, 5% betel leaf extract showed better healing on 7th and 15th day of wounds. This finding supports current study in which betel leaf extract showed increased angiogenesis and better wound healing compared to control group however at lesser extent than topical zinc oxide. Another study¹⁴ found that there was an increase in angiogenesis, along with new fibroblasts and macrophage formation, thus it further supports our study. The same study found that using betel leaves will lead to a significant rise in hydroxyproline content, superoxide dismutase (SOD) levels, and a decrease in the amount of malondialdehyde levels.¹⁴ A systemic review¹² was able to confirm that betel leaf has an effect on increase in blood vessel growth, collagen layer thickening, amount of epithelialization, fibrous tissue formation, weight of granulation tissue, hydroxyproline content, and ultimately inhibits bacterial cell growth.¹² These findings along with ours can confirm that betel leaf causes increase in the number of blood vessel formation, but also improves wound healing in many other ways. Betel leaf has the ability to suppress the inflammation and promote an increase in re-epithelialization.¹⁷ Suharto *et al*¹⁸ showed that betel leaf extract when applied topically enhanced wound healing and wound closed early when applied in higher concentration. They expressed that inflammatory process starts within 24 hours of wound occurrence. Fibroblasts and vascular endothelial cells begin to proliferate and form new granulation tissue which is a significant sign of healing. This is in accordance with our study where new blood vessels formed rapidly after applying betel leaf extract.

Betel leaf can be further studied by comparing different types of betel leaves such as red betel leaf or piper betel leaf.¹⁹ Future studies can also be used to explore other agents that are being currently tested in trials regarding their efficacy on wound healing and be compared to betel leaf extract.

CONCLUSION

Topical application of zinc oxide was found superior in wound healing by increasing angiogenesis on all days of sampling compared to betel leaf extract. Topical application of zinc oxide is effective for facilitating wound healing. Betel leaf extract can be considered as a suitable alternative to zinc oxide.

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SH: Formal analysis and proof reading and final approval

AZ: Statistical analysis and critical review

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