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Wound Healing Potential of *Strobilanthes alternata* Root Extract in Excision and Thermal Injury Models in Albino Swiss Mice

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ABSTRACT

Aims and Objective: Herbal plants are increasingly recognized in wound healing due to their multi-targeted effects, biocompatibility, safety profiles, and efficacy. The current work explores the potential for healing wounds of *Strobilanthes alternata* ethanolic extract from roots using excision and thermal wound models in albino Swiss mice.

Materials and Methods: After acclimatization, the experimental animals were randomly divided into the different groups, and a model was made with the excision wound and the thermal wound. The disease control kept as an untreated, whereas the treatment involved topical applications of different dosages of plant extracts in the treatment groups. A standard treatment group was applied by the mupirocin ointments. After administering the treatment to respective groups, the animals were continuously observed for wound contraction, closure rate, and epithelialization. At the end of the experiments, subsets of CBC, antioxidant enzymes, and the levels of pro-inflammatory cytokines were observed.

Results: It demonstrated the dose-dependent acceleration of wound contraction over time in both models, increased the levels of antioxidant enzyme (SOD) activity in the treatment groups, and a significant decrease in cytokines (IL-6 and IL-1β). No changes in CBC subsets were observed; with the exception of platelets ($p<0.001$), all results exhibited $p>0.05$ between the groups. The efficacy of the extract in epithelialization, remodeling/proliferation, and inflammation reduction was observed in histological studies.

Conclusion: The findings support the ethnomedicinal use of *S. alternata* and support its possibility as a cost-effective and natural type of wound-healing agent.

Keywords: Antioxidants, Cytokines, Excision, Herbal Plants, Wound Healing, Thermal wound.

INTRODUCTION

Any damage sustained on a body is termed a wound, and this is usually a breach of the epidermis component of the skin, which interferes with the normal structure and functioning of the skin. Wounds are classified as open or closed based on their cause and physiological response and as acute or chronic wounds based on the process of healing. Normal wounds, surgical incisions, and accidental injuries (burns, chemical, electrical, or thermal trauma) belong to this category of acute wounds and progress through the typical stages of inflammation and tissue growth and remodeling and have a relatively predictable healing

time. However, in contrast to chronic wounds, the healing being a by-product of these processes is aberrant and normally ineffective because of either local infection or other complications, causing delayed healing. [1] The healing of wounds is one of the most complex processes in the human body. It involves the coordination in time and space of various cell species engaged in hemostasis, inflammation, proliferation, regeneration, and remodeling. [2]

Hemigraphis alternata (or *Strobilanthes alternata*), commonly called metal leaf or Java ivy, is a low, biennial shrub native to Malaysia and usually growing to 1520 cm. It has also been available in other parts, such as India and Bangladesh. It has numerous phytoconstituents present in the plant, such as proteins, steroids, tannins, carbohydrates, flavonoids, alkaloids, triterpenes, amino acids, and phenols. *S. alternata* has had a long history of being used in the medicinal systems because of its positive effects on treatment, particularly on wound healing. Its leaves have been used to rub wounds and injuries to induce healing and prevent bleeding. It has also been employed in the treatment of hemorrhoids, gallstones, sexually transmitted diseases, menorrhagia, as well as anemia, and kidney stones in folk medicine. [3][4] The extracts and juice of *S. alternifolia* leaves have been noted to enhance faster wound healing and thus have an antimicrobial and an anti-inflammatory effect. It is an ethnomedicinal plant of high pharmacological value due to the presence of bioactive compounds that are important in regulating the wound healing phases. [4] Excision and thermal wound models are common experimental models in wound healing studies and evaluation of therapeutic agents. In excision, a section of skin can be removed surgically, and natural healing observed, such as wound contraction, epithelialization, and collagen deposition. Conversely, the thermal wound model entails wounding a thermal injury through the use of controlled heat exposure, providing an insight into the healing process of burn wounds that includes tissue necrosis, inflammation, and slow regeneration. Both models can be useful in assessing the effectiveness of wound-healing therapy and in the interpretation of various physiological reactions to pathologic injury. Using herbal plants for wound healing is a well-established practice rooted in traditional medicine systems like Ayurveda, Traditional Chinese Medicine (TCM), and Unani. The rationale behind using herbal plants for wound healing includes scientific, traditional perspectives and phytochemical constituents with therapeutic effects due to their containing a wide range of bioactive compounds, such as flavonoids and tannins. Saponins Alkaloids Terpenoids and phenolics These compounds aid various phases of wound healing: hemostasis, inflammation, proliferation, and remodeling.

MATERIALS AND METHODS

Plant collection and authentication: The plant materials (roots of *Strobilanthes Alternata*) were collected from the authenticated vendor during April month, Bengaluru, India. They were authenticated by Dr. V. Rama Rao, Research Officer (Botany), Central Ayurveda Research Institute, and Reg. No. SMPU/CARI/BNG/2025-26/357.

Preparation of Extraction: *Strobilanthes alternata* was rinsed in tap water. Drying at room temp. after being cut into slices. The dried roots were pulverized, and 100 g of this material was extracted with 1000 mL ethanol in 3 cycles in a condenser and refluxed 3 to 4 hours per cycle until the amount was reduced by half. The extract was then filtered, dried at room temperature, and kept at 2 to 8 degrees Celsius until usage.

Dose Selection: The doses were selected based on earlier studies conducted by Rahman *et al.* (2019). Therefore, 200 and 400 mg/kg b.w. were chosen in the current work. [3]

Experimental Design

Swiss albino male mice weighing 20–25 g was used. The animals were housed in standard protocols, which included a 12-hour light and 12-hour dark cycle, relative humidity of 50% ± 5%, and ambient temperature of 22 ± 2°C. The animals were kept for acclimatization for five days before the experiment in a controlled environment. [5,6] The protocol was duly approved by IAEC and Sl.no. KCP-IAEC/16/24-25/12/10/03/25

Groupings:

The animals were divided with the following manners; (n = 6/each group)

1. Normal control – Received normal saline
2. Excision Control – No treatment
3. (Excision) Std drug - Mupirocin ointment 2%, q.s. topically
4. (Excision) Test drug – *Strobilanthes alternata* 400 mg/kg, q.s. topically
5. (Excision) Test drug – *Strobilanthes alternata* extract 200 mg/kg, q.s. topically
6. Thermal Control – No treatment
7. (Thermal) Test drug – *Strobilanthes alternata* 400 mg/kg, q.s. topically
8. (Thermal) Test drug – *Strobilanthes alternata* 200 mg/kg, q.s. topically

Wound Induction model

Excision Wound model:

The animal was shaved prior to use for the excision wound. The animals were anesthetized with the low dose of phenobarbital sodium. Using toothed forceps, a surgical blade, and pointed scissors, a full thickness of the excision wound measuring 2.5 cm in width and 0.2 cm in depth was made, and the wound remained exposed. After 24 hrs. the wound and the respective treatment were applied over time. [6]

Thermal wound model:

With slight modification of the model mentioned in the *Walker-Mason method*, the animals were shaved (1.5cm^2 on dorsal surface) prior to use for thermal induction. After stabilization, the animals were anesthetized with a light dose of phenobarbital (approx. 40 mg/kg b.w.), and a metal rod (10 mm) was heated for 5 to 10 sec. and placed on the dorsal shaved surface of the mice for 5 sec. After 24 hours of induction, the animals were used for intervention of the respective treatment**.** [7]

From the time of wound induction until full healing, the animals were monitored carefully; wound closure was monitored throughout the time. At the end of the experiment the animals were anesthetized for blood withdrawal from the retro-orbital for hematological assessment (Hb, RBC, TLC, Neutrophils, Lymphocytes, Platelets and NLR). Later the animal was sacrificed by using the phenobarbital sodium, the skin was isolated, and a portion of skin was homogenized with the cold PBS and stored at -20°C for biochemical assessments; Antioxidant (SOD enzyme activity) [8] and Proinflammatory cytokines (IL6 and Il-1β). [9,10] Another half of the skin was kept in 10% formalin for histological assessment.

Statistical Analysis

The results were assessed Mean ± SEM with n = 6/group. An analysis of the data was done with GraphPad Prism statistical software. The importance of each variation among the groups was determined using ANOVA followed by Tukey post hoc test. The differences between the control (untreated) group and all other groups were tested, and $p < 0.05$ was considered significant.

RESULTS

Wound Closure rate over time for different groups

Figures 1 and 2 show the wound contraction (in mm) over time for each group. Excision groups treated with *Strobilanthes alternata* 400 mg/kg show the fastest and most complete wound contraction at 400 mg/kg. Strobilanthes alternata shows complete healing by Day 16, which is comparable to STD drug treatment (mupirocin). Thermal control shows the slowest and incomplete wound contraction (below 50%) by Day 16. *Strobilanthes alternata* (400 mg/kg) is highly effective in excision wounds, comparable to or even superior to mupirocin. Thermal wounds are difficult to treat, but the test drug still improves healing notably. Control groups validate the healing effects observed in treated groups. Unfortunately, there were no animals left in standard treatment groups to compare.

Hematological subsets assessments

Table 1 shows the CBC subsets. The Hb levels across groups are relatively close (ranging from 9.9 to 13.6 g/dL) and shown to be non-significant across the treatment groups ($p > 0.05$). RBC Count Highest in Excision Test formulation 400 mg/kg (9.47 mil/µL), suggesting strong systemic support for healing. Total Leukocyte Count (TLC) elevated in Thermal Test formulation 400 mg/kg (4.0 ± 0.23), possibly reflecting acute immune activation. Suppressed in Excision Test formulation 400 mg/kg (1.6 ± 0.09), which may indicate reduced inflammation and faster healing. Thermal Test 200 mg/kg shows the highest neutrophil count (15.6%), indicating active immune engagement. Lymphocytes peaked in the thermal test formulation at 400 mg/kg (94.3%) and the excision test formulation at 200 mg/kg (91.9%), possibly indicating a shift toward tissue repair or regeneration. Platelet Count Highest in Excision Test 400 mg/kg (1,483 x10^3/µL), essential for clotting and tissue repair. Lowest in Excision Test 200 mg/kg (816 ± 47), though still within a functional range. Normal control was showing baseline, and the excision test formulation at the dose of 200 mg/kg has the lowest NLR (~0.058), indicating minimal inflammatory burden. The thermal test formulation at the dose of 200 mg/kg shows the highest NLR (0.207), suggesting a more activated inflammatory state. The thermal test formulation at the dose of 400 mg/kg surprisingly shows the lowest NLR (0.036), suggesting effective modulation of inflammation at a higher dose.

Antioxidant enzyme activity (SOD) in skin tissue homogenate supernatant

Figure 3 shows the thermal test of the SA 400 mg/kg group shows the highest SOD activity (20.85 ± 1.21), indicating strong antioxidant potential. Excision Control has the lowest SOD activity (8.40 ± 0.49), suggesting oxidative stress due to lack of treatment. Excision and thermal test groups with SA (*Strobilanthes alternata*) show dose-dependent increases in SOD activity. These findings highlight the efficacy of *SA* in enhancing antioxidant defenses, which may mitigate oxidative damage.

Proinflammatory cytokines (IL-6 and IL-1β) in skin tissue homogenate supernatant

Figure 4 shows cytokine levels for different groups. Normal control maintains baseline cytokine levels, as expected. The excision control group shows the highest levels of IL-6 and IL-1β, indicating elevated inflammation in untreated wounds. Excision Test SA 400 mg/kg significantly reduces both IL-6 and IL-1β, showing strong anti-inflammatory potential. The Thermal Test SA 400 mg/kg also lowers cytokines, but not as strongly as the Excision Test 400 mg/kg group.

Assessment study with pictographs

The pictogram image (figure 5) analysis over time reflects significant differences in healing outcomes between excision and thermal wound models, as well as the therapeutic effectiveness of the SA extract at different doses. All groups began with a comparable wound size on day 0. In the excision, the untreated control group showed the slowest rate of wound closure, indicating limited natural healing. In contrast,

the standard treatment group (Mupirocin 2%) and the SA extract at 400 mg/kg demonstrated markedly faster healing, with nearly complete wound closure by day 16. The SA extract at 200 mg/kg also facilitated substantial wound contraction, though at a slightly slower rate than the higher dose, indicating a dose-dependent response. In the thermal wound model, the control group showed the poorest healing, with minimal reduction in wound size over the 16-day period, consistent with the greater severity and deeper tissue damage associated with thermal injury. However, treatment with the SA extract improved healing outcomes in a dose-responsive manner. The 400 mg/kg dose significantly enhanced wound contraction compared to the control, while the 200 mg/kg dose showed moderate improvement but not comparable as excision model.

Histological examination evaluation

The figure 6 and table 2 show the H&E staining compares the histological data amongst various treatment groups in an excision and thermal wound model. In NC the skin morphology is intact, and there is normal healthy collagen and dermal matrix, no bacterial colonies, and no inflammation in the dermis or beneath the muscle layer. Contrastingly, the excision disease control (DC) group exhibits extreme tissue destruction, bacteria colonies at the periphery of the wound, necrotic epidermis and dermis, and aggregates of neutrophils in the dermis, ultimately on the commode of the wound. There is also the presence of some neutrophils below the level of the muscular tissue, which signifies focal inflammation. The standard excision group (STD) shows marginal necrosis of the epidermis, a reconstituting dermis narrowly and fibrotically remodeling, and neutrophilic clumps at the margin of the wound, but no inflammation in the dermis. Moderate inflammation and edema are found under the muscle layer. The excision testing group appears vastly improved with an essentially normal epidermis with evidence of re-epithelialization, a thick dermis indicating a fibrous repair, and a lack of dermal inflammation. Nonetheless, acute inflammation and a strong immune reaction are observed in the profound layers of muscles. The most pronounced histological damages can be found in the thermal disease control group (DC Thermal), which evinces bacterial colonies, wide re-epithelialization with enormous epidermis sloughing, and disintegration of the dermal connective tissue matrix. They also exhibit much neutrophilic press infiltration in the wound margin and inflammation that expands under the muscle layer with edema. The thermal model test group proves to have slight improvement, where few bacterial colonies can be found, a small necrosis of the epidermis, and partially regenerated or dissolved dermal connective tissue. There is inflammation in the dermis and at the wound margin, though it contains neutrophilic aggregates, but there is acute inflammation with edema and focal hemorrhage in the muscle layer.

Figure 6 and table 3 give an understanding of the level of tissue repair, collagen deposition, and fibrosis among the various wound models used in the experiment and the various treatment groups. Tissues of the normal control (NC) group contained a normal, organized arrangement of the collagen fibers, no granulation tissue, no fibrosis, and no damaged intact muscle structures, indicating healthy, densely mature tissues without any pathological alterations. In the DC (Excision) group, obvious disorganized collagen around the wound edge, thick granulation tissue, blue collagen resembling fibrosis, destruction of normal muscle architecture, and immature fibrotic tissue were noted, implying poor healing and chronic inflammation. The STD (Excision) group had moderate collagen partially aligned, moderate granulation tissue, mild to moderate fibrosis, slightly affected muscle integrity, and tissue seemingly at a granulating-maturing transition, indicating moderate repair. The test group under excision wounds demonstrated the most positive findings, additional collagen deposition, and improved alignment, indicating an early remodeling process, robust vascularized granular tissue, less fibrosis, signs of early muscle regeneration,

and clear tissue remodeling, which ironically demonstrates effective healing. The tissue in the DC (Thermal) group had dense fibrotic bands, fibrotic and irregular granulation tissue, widespread fibrosis indicative of intense blue staining, thermally destroyed muscle, and poorly supported and remodeled immature tissue, a characteristic sign of excessive tissue loss. Conversely, the test thermal group exhibited moderate collagen with partial remodeling, moderate granulation with formation of capillaries, focal fibrosis, partial maintenance of muscle with signs of repair, and a combination of granulation and early remodeling, which indicates some healing, but not as complete as it is evident in the excision test group.
Figure 7 shows the differences in the effects of excision and thermal wound models on tissue damage and healing are reflected in the data in the histopathological scoring. The disease control (DC) group demonstrated a high total score of 27, highly indicating tissue damage and insufficient healing even in the excision model. The standard (STD) excision group also revealed a lower score of 17, reflecting moderate tissue alteration, whereas the test group revealed a notably low score of 9, implying minimal damage and an excellent tissue repair mechanism, thus espousing the effectiveness of the test treatment in enhancing tissue recovery. Conversely, the thermal wound model turned out to be more significant. The total score of 28 in the thermal DC group was the highest, showing a massive thermal injury with severe inflammation and tissue interruption. The thermal wounds test group scored 17, indicating a moderate level of obtaining some degree of healing with diminished levels of inflammation and fibrosis but less successful compared to the excision model. In general, these data provide evidence that thermal injuries are destructive and more challenging to repair than excision wounds. The test treatment had a superior healing effect on excision-induced wounds and a moderate protective effect during thermal injury; therefore, the test treatment has possible therapeutic potential in wound management.

DISCUSSION

The present study evaluated the wound healing efficacy of *Strobilanthes alternata* root extract using excision and thermal wound models in Swiss albino mice. Wound healing is a dynamic process involving overlapping phases of inflammation, proliferation, and remodeling; interruption at any stage can result in delayed or chronic wounds. [11] The findings of this work suggest that the extract accelerates healing by targeting multiple mechanisms relevant to these phases.
Available phytochemical reports on *S. alternata* indicate abundant phenolics, flavonoids, terpenoids, and alkaloid classes with well-documented anti-inflammatory, antioxidant, and antimicrobial actions relevant to each healing phase. [12, 13] The observed reductions in IL-6 and IL-1β are consistent with polyphenol-mediated suppression of NF-κB signaling, facilitating resolution of inflammation and progression to proliferation. The eugenol archetype (a phenol abundant in *Syzygium aromaticum*) down-modulates NF-κB and pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) in diverse models, aligning with our cytokine data. [14–16] Elevated SOD activity in treated groups indicates strengthened redox defense. Controlled ROS is pro-healing, but excess oxidative stress prolongs inflammation and impairs re-epithelialization; antioxidant reinforcement (for example., via phenolics/flavonoids) restores balance and supports tissue formation. [17–19] Histology showing improved collagen alignment and earlier remodeling (notably at 400 mg/kg) agrees with literature linking phenolic-rich botanicals to increased hydroxyproline content, fibroblast proliferation, and organized granulation tissue. [20] Reduced bacterial burden in SA-treated wounds (H&E) aligns with the broad-spectrum activity reported for phenolic/terpenoid-rich extracts against wound colonizers such as Staphylococcus aureus and Pseudomonas aeruginosa; clove (eugenol-

rich) serves as a well-studied comparator showing load reduction and faster closure in infected-wound models. [21-23]

Collectively, these mechanistic links explain our findings: faster wound contraction in the excision model and partial but evident benefits in thermal injury, likely via dampened inflammation/oxidative stress, infection control, improved granulation, and collagen maturation. Our data are concordant with recent work positioning *H. alternata* as a wound-healing botanical across in vitro scratch assays and in vivo models, including molecular evidence for pro-healing gene modulation (for example., TGF-β1).[24] Parallel evidence from *S. aromaticum* confirms that eugenol-rich extracts shorten time to closure, raise hydroxyproline, and enhance granulation in excision and infected wounds, including difficult-to-heal contexts (for example that., diabetic ulcers). [20–22] The shared phenolic signature across these species provides a plausible chemical rationale for the comparable biological readouts observed here.

This study demonstrates the dose-dependent wound-healing efficacy of *S. alternata* root extract across both excision and thermal models, with convergent biochemical, histological, and microbiological evidence highlighting it as a multi-target healing candidate. Key strengths include the dual-model design, integration of multiple outcome measures (biochemistry, histology, and infection control), and benchmarking against mupirocin in excision wounds. Limitations include the use of a crude extract without isolation of active fractions, lack of long-term scar or tensile strength assessments, absence of angiogenesis quantification, and the unavailability of a standard reference for thermal wounds, which restricted direct comparison beyond the respective groups. Future studies should focus on bioassay-guided fractionation, mechanistic pathway analysis (NF-κB, Nrf2, TGF-β, VEGF), and use of contaminated wound models with quantitative microbiology and angiogenesis markers.

CONCLUSION

In conclusion, the *Strobilanthes alternata* extract demonstrates promising wound-healing efficacy. The wound contraction over time and % closure rate, biochemical analysis (SOD, IL6 and IL-1β), and combined histopathological and trichrome staining demonstrate that the test formulation containing *Strobilanthes alternata* extract exhibits significant wound healing potential. Excision wounds responded more favorably to treatment, with evidence of enhanced re-epithelialization, organized collagen deposition, reduced fibrosis, and early tissue remodeling, especially at the higher dose (400 mg/kg). Thermal wounds, while more severe and slower to heal, also showed improvement with treatment, including reduced inflammation, moderate collagen alignment, and partial tissue regeneration.

REFERENCES

1. Yazarlu O, Iranshahi M, Khayat Kashani HR, Reshadat S, Habtemariam S, et al. Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. Pharmacol Res. 2021;105841. doi:10.1016/j.phrs.2021.105841.
2. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. Physiol Rev. 2019;99(1):665–706. doi:10.1152/physrev.00067.2017.
3. Mushiur Rahman SM, Atikullah M, Islam MN, Mohaimenul M, Foysal A, et al. Anti-inflammatory, antinociceptive and antidiarrhoeal activities of methanol and ethyl acetate extract of Hemigraphis alternata leaves in mice. Clin Phyto. 2019;5(1):1–11. doi:10.1186/s40816-019-0110-6.

4. Devasahayam AB, Palollathil A, Kumar ST, Jaikanth C, Shubham SU, Sakshi SP, et al. Role of Hemigraphis alternata in wound healing: metabolomic profiling and molecular insights into mechanisms. Sci Rep. 2024;14:3872.
5. Quazi A, Patwekar M, Patwekar F, Mezni A, Ahmad I, Islam F. Evaluation of wound healing activity (excision wound model) of ointment prepared from infusion extract of polyherbal tea bag formulation in diabetes-induced rats. Evid Based Complement Alternat Med. 2022;2022(2):7.
6. Das K. Wound healing potential of aqueous crude extract of Stevia rebaudiana in mice. Rev Bras Farmacogn. 2013;23(2):351–7.
7. Mehrabani M, Seyyedkazemi SM, Nematollahi MH, Jafari E, Mehrabani M, et al. Accelerated burn wound closure in mice with a new formula based on traditional medicine. Iran Red Crescent Med J. 2016;18(11):e26613.
8. Ware CF, Santee S, Glass A. Tumor necrosis factor-related ligands and receptors. In: Thompson A, editor. San Diego (CA): Academic Press; 1998.
9. Van Snick J. Interleukin-6: An overview. Annu Rev Immunol. 1990;8(1):253–78.
10. Beyaert R, Fiers W. Tumor necrosis factor and lymphotoxin. In: Cytokines. Elsevier; 1998. p. 335–60.
11. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Braz J Pharmacogn. 2009;19(1B):212–7.
12. Chandra G, Singh M, Gupta N, Verma RK. Role of Hemigraphis alternata in wound healing: Metabolomic and bioactivity perspectives. BMC Complement Med Ther. 2024;24:26.
13. Rahman M, Hossain MM, Saha S. Ecological and therapeutic insights into Strobilanthes alternata. Asian J Pharm Res Dev. 2024;12(1):1-12.
14. Shalaby MA, Mohamed AA, El-Tantawy WH, Ahmed OM. The healing capability of clove flower extract in diabetic foot ulcers. Plants. 2022;11(8):1060.
15. Lima RS, Cardoso M, Carvalho JF, Souza LM, Nogueira PC, Moura D, et al. Ortho-eugenol exhibits anti-nociceptive and anti-inflammatory activities via NF-κB and p38 MAPK. Immunopharmacol Immunotoxicol. 2017;39(1):1-9.
16. García-Serrano AM, Medina-Luna D, Guzmán-Gutiérrez SL. Anti-inflammatory and antioxidant potential of eugenol: An overview. Antioxidants. 2018;7(10):156.
17. Serena TE, Bohn G, Niezgoda J, Snyder RJ. The role of antioxidants on wound healing. Antioxidants. 2021;10(2):205.
18. Kim J, Lee H, Park H, Choi S. Reactive oxygen species and antioxidants in wound healing. Biochem Biophys Rep. 2025;38:101447.
19. Muñoz-Mármol AM, García-García F, Sánchez-Esteban M, Cañueto J. Cellular and molecular roles of ROS in wound healing. Commun Biol. 2024;7:532.
20. Abdel-Rahman SM, El-Desoky AM, Ahmed WS. Antibacterial activity of Syzygium aromaticum bud oil and its wound-healing efficacy in rats. Antibiotics. 2022;11(12):1790.
21. Basmacioglu-Malas O, Tuncel NB, Sari M, Akgul M, Bilgin M. Comparative antibacterial activity of clove extract against Pseudomonas aeruginosa. BMC Complement Med Ther. 2024;24:105.
22. Zhang Y, Li X, Chen H, Wu P, Zhou Q. Effects of clove extract on antibacterial activity against wound pathogens. J Dairy Sci. 2025;108(5):1-12.

23. Various authors. Systematic notes on eugenol-mediated granulation/collagen improvements in rat skin: Selected experimental reports and histomorphology, 2019–2020. ResearchGate [Internet].
24. Kumar A, Singh V, Sharma R. Wound-healing potency of Hemigraphis alternata leaf extract with molecular evidence. ResearchGate [Preprint]. 2021.

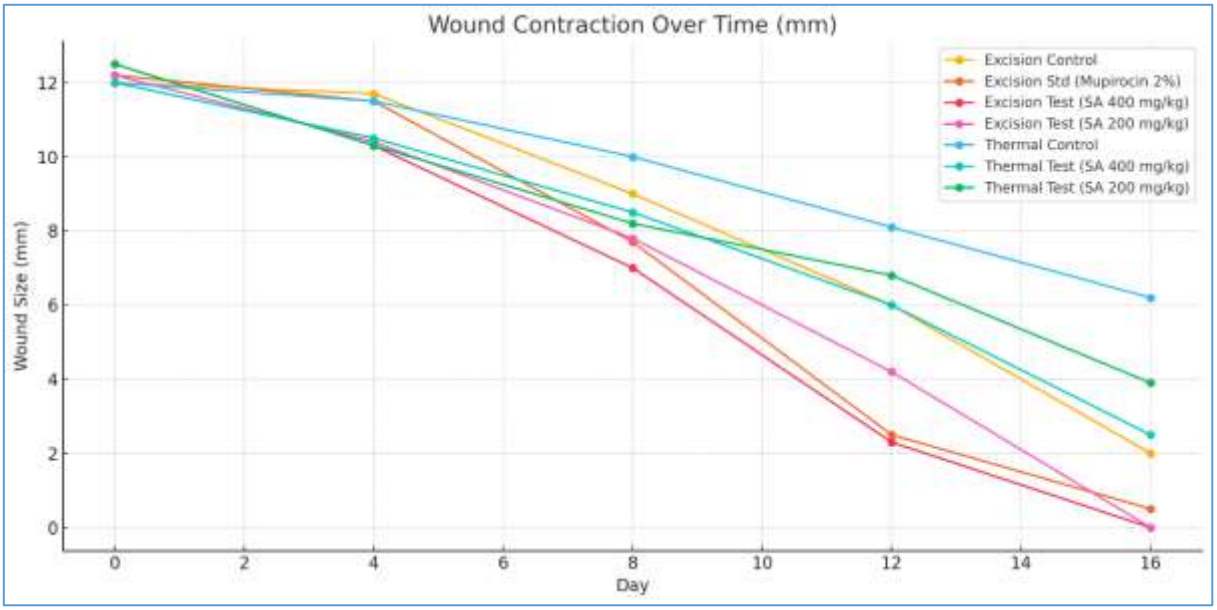


Figure 1: Wound Closure rate over time for different groups. Values were expressed as Mean ± S.E.M, n = 6

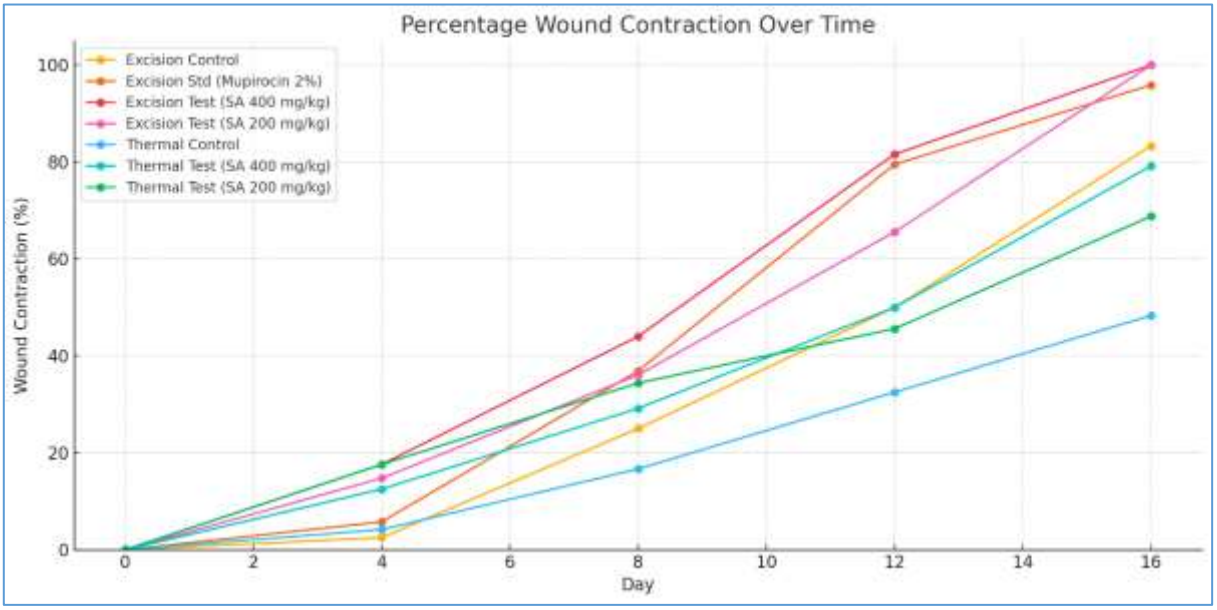


Figure 2: % Wound contraction over time. Values were expressed as Mean ± S.E.M, n = 6

Parameter	Normal Control	Excision Control	Excision Std-Mupirocin 2%	Excision Test-SA 200 mg/kg	Excision Test-SA 400 mg/kg	Thermal Test-SA 200 mg/kg	Thermal Test-SA 400 mg/kg
Hemoglobin (g/dL)	9.9 ± 0.57	12.3 ± 0.71	13.6 ± 0.79	12.0 ± 0.70	11.0 ± 0.64	13.4 ± 0.78	13.1 ± 0.76
RBC (mil/µL)	6.84 ± 0.40	7.67 ± 0.45	8.17 ± 0.47	7.56 ± 0.44	9.47 ± 0.55	8.16 ± 0.47	7.73 ± 0.45
TLC (x10³/µL)	3.8 ± 0.22	2.3 ± 0.13	3.7 ± 0.21	3.2 ± 0.19	1.6 ± 0.09	3.2 ± 0.19	4.0 ± 0.23
Neutrophils (%)	5.3 ± 0.31	12.0 ± 0.70	9.8 ± 0.57	5.2 ± 0.30	10.0 ± 0.58	15.6 ± 0.90	3.4 ± 0.20
Lymphocytes (%)	90.8 ± 5.26	85.5 ± 4.94	85.7 ± 4.97	91.9 ± 5.31	85.8 ± 4.97	75.4 ± 4.37	94.3 ± 5.47
Platelets (x10³/µL)	999 ± 58	1,224 ± 71	1,320 ± 77	816 ± 47	1,483 ± 86	999 ± 58	990 ± 57
NLR	0.06 ± 0.002	0.14 ± 0.005	0.11 ± 0.004	0.06 ± 0.002	0.12 ± 0.00	0.21 ± 0.008	0.04 ± 0.001

Table 1: CBC subsets– Values were expressed as Mean ± SEM (n = 3). Note: Except platelets p<0.001 all were showing non-significant p>0.05 between the groups.

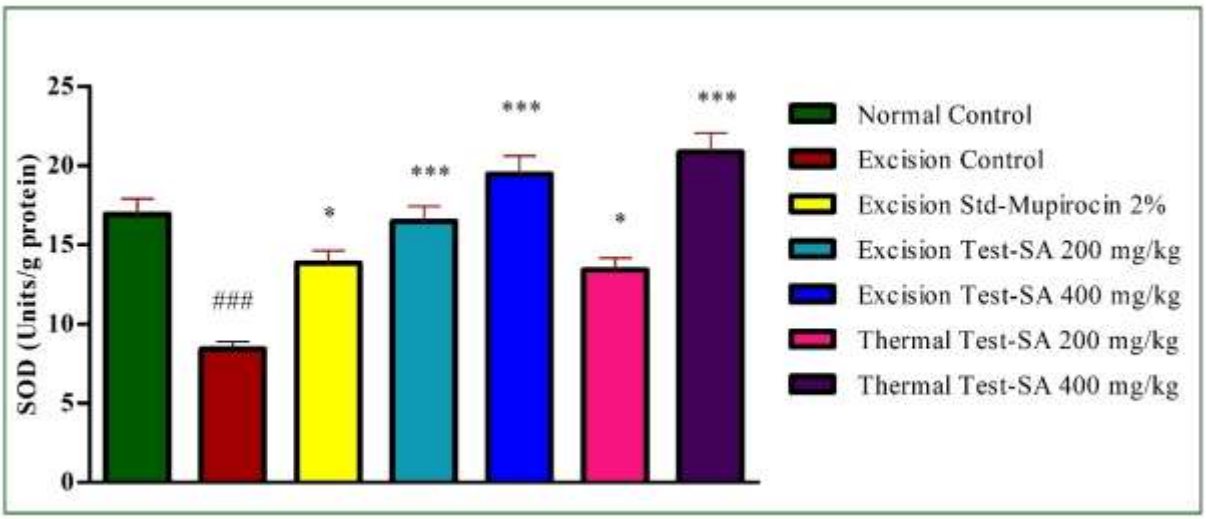


Figure 3: SOD levels for different groups. Values were expressed as Mean ± SEM (n = 3), ###p<0.001 compared to normal control, *p<0.05, **p<0.01, and ***p<0.001 compared to disease control (Excision control)

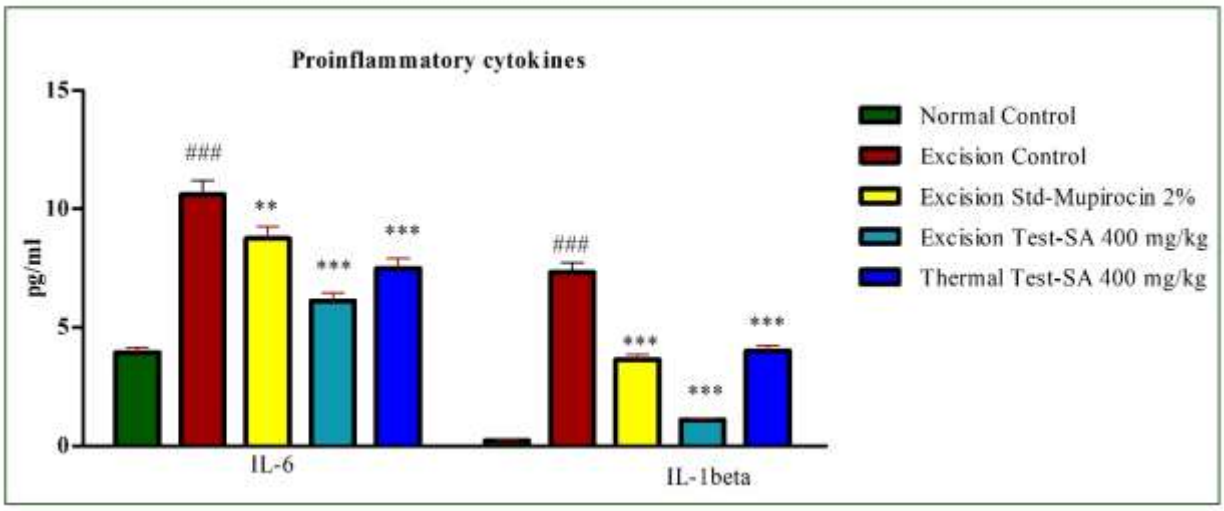
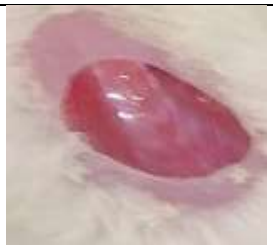
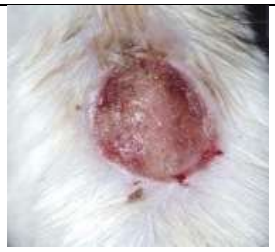
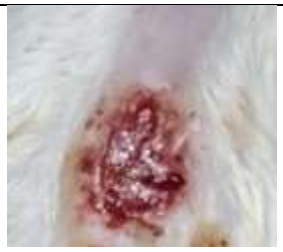
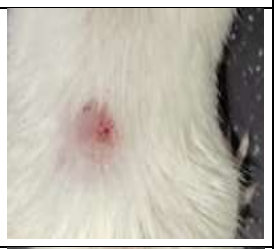


Figure 4: Proinflammatory cytokines. Values were expressed as Mean ± SEM (n = 3), ###p<0.001 compared to normal control, **p<0.01 and ***p<0.001 compared to disease control (Excision control)

Pictographically images over time for different groups

Groups/Day	**0**	**5**	**10**	**15**
Excision Control				
(Excision) Std drug - Mupirocin ointment 2%				
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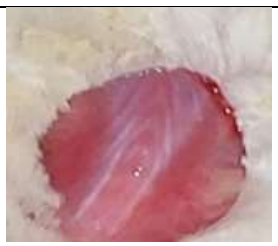
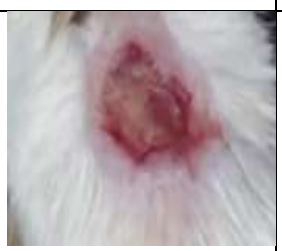
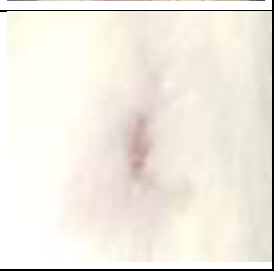
(Excision) Test drug – *SA* extract 200 mg/kg, q.s. topically				

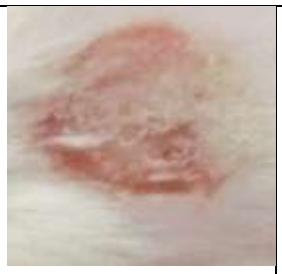
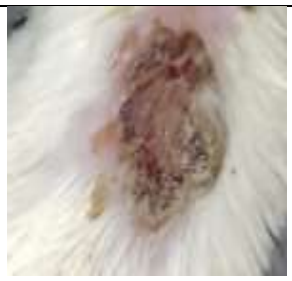
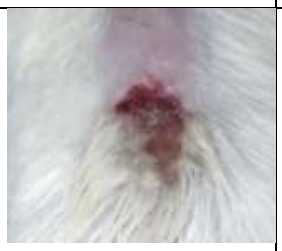
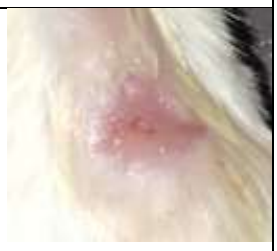
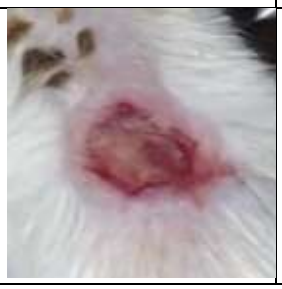
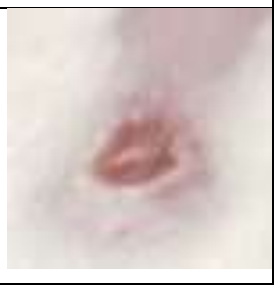
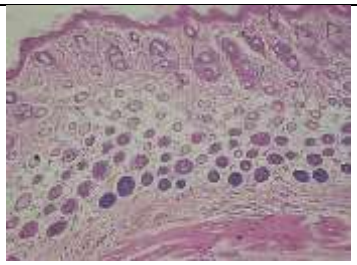
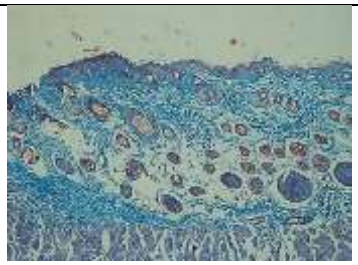
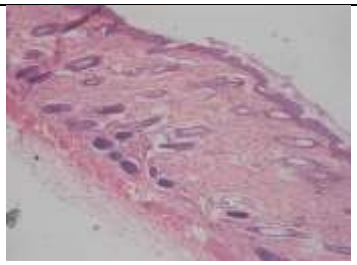
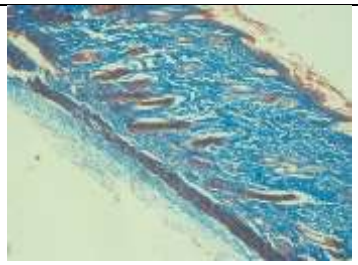
Thermal Control				
(Thermal) Test drug – *SA* 400 mg/kg, q.s. topically				
(Thermal) Test drug – *SA* 200 mg/kg, q.s. topically				

Figure 5: Pictorial image of wound closure rate for different groups

Histology: Skin tissue

Figure 6: Shows Skin tissue histological assessments

Groups	**H&E staining image, Transaction, Magnification 10X**	**Trichrome-stained Image, Magnification 10X**
Normal Skin		
Excision Control		

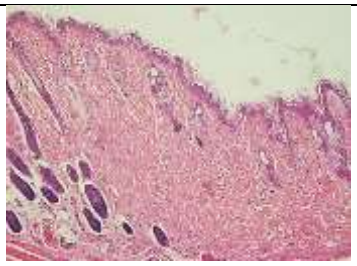
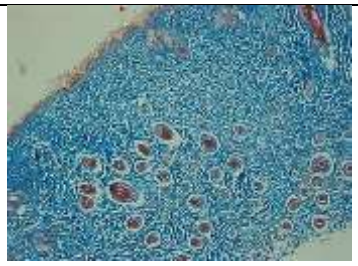
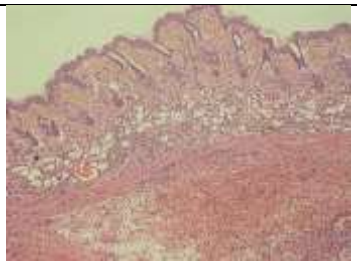
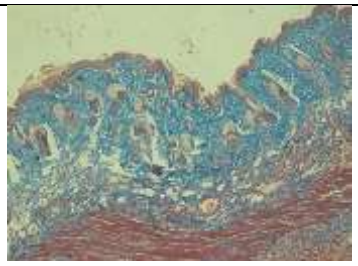
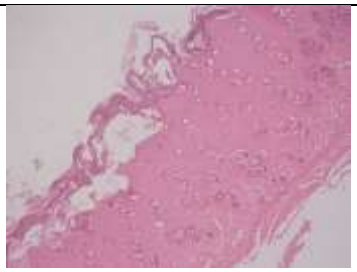
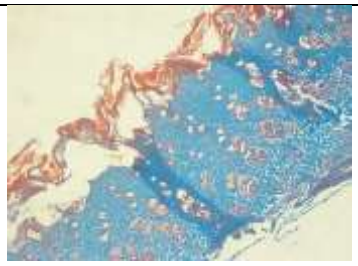
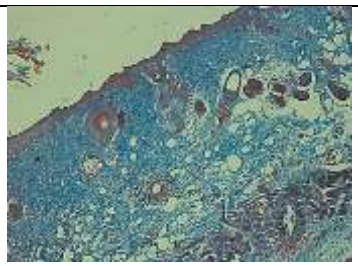
(Excision) Std drug - Mupirocin ointment 2%		
(Excision) Test drug – *SA* 400 mg/kg, q.s. topically		
Thermal Control		
(Thermal) Test drug – *SA* 400 mg/kg, q.s. topically		

Table 2: H&E staining Report Summary

Parameter	NC (Normal Control)	DC (Excision)	STD (Excision)	Test (Excision)	DC (Thermal)	Test (Thermal)
Bacterial Colonies	Absent	Present at wound margin indicating potential infection risk	Absent	Absent	Present	Few colonies in wound margin
Epidermis	Intact stratified squamous epithelium	Necrotic	Marginal necrosis	Normal — re-epithelialization may have begun	Severe thermal damage; sloughing of epidermis	Mild necrosis

Dermis	Healthy collagen and dermal matrix	Necrotic	Thin dense pink dermis fibrotic remodeling	Thin dense dermis — suggestive of fibrous healing	Destruction of dermal connective tissue matrix	Partially regenerated or degraded connective tissue
Inflammation (Dermis)	No inflammation	Neutrophilic aggregates in deep dermis at wound margin	Neutrophilic aggregates at wound margin, but no inflammation in dermis	No inflammation in dermis	Neutrophilic aggregates at wound margin	Neutrophilic aggregates at wound margin, no dermal inflammation
Inflammation Below Muscle Layer	No inflammation	Some neutrophils	Mild inflammation and oedema	Acute inflammation — marked immune response in deeper layers	Inflammation and oedema	Acute inflammation with oedema and focal hemorrhage

Table 3: Trichome Staining Report Summary

Parameter	**NC (Normal Control)**	**DC (Excision)**	**STD (Excision)**	**Test (Excision)**	**DC (Thermal)**	**Test (Thermal)**
Collagen Deposition	Normal, organized collagen fibers	Excessive disorganized collagen at wound edge	Moderate collagen, partial alignment	Increased collagen with better alignment — early remodeling	Dense fibrotic bands	Moderate collagen with partial remodeling
Granulation Tissue	Absent	Present with dense matrix	Moderately present	Present, well-vascularized	Present, fibrotic and irregular	Moderate granulation and capillary formation
Fibrosis Severity	None	Dense blue collagen	Mild to moderate	Signs of remodeling with reduced fibrosis	Extensive blue staining indicating advanced fibrosis	Focal fibrosis with partial healing

Muscle Integrity	Preserved	Loss of normal muscle structure due to excision	Slightly altered	Early muscle regeneration	Thermal destruction of muscle layer	Partial preservation with signs of repair
Tissue Maturity/Remodeling	Normal mature tissue	Immature fibrotic tissue	Progressing from granulation to maturation	Remodeling evident	Immature, fibrotic, poorly remodeled tissue	Mixture of granulation and early remodeling

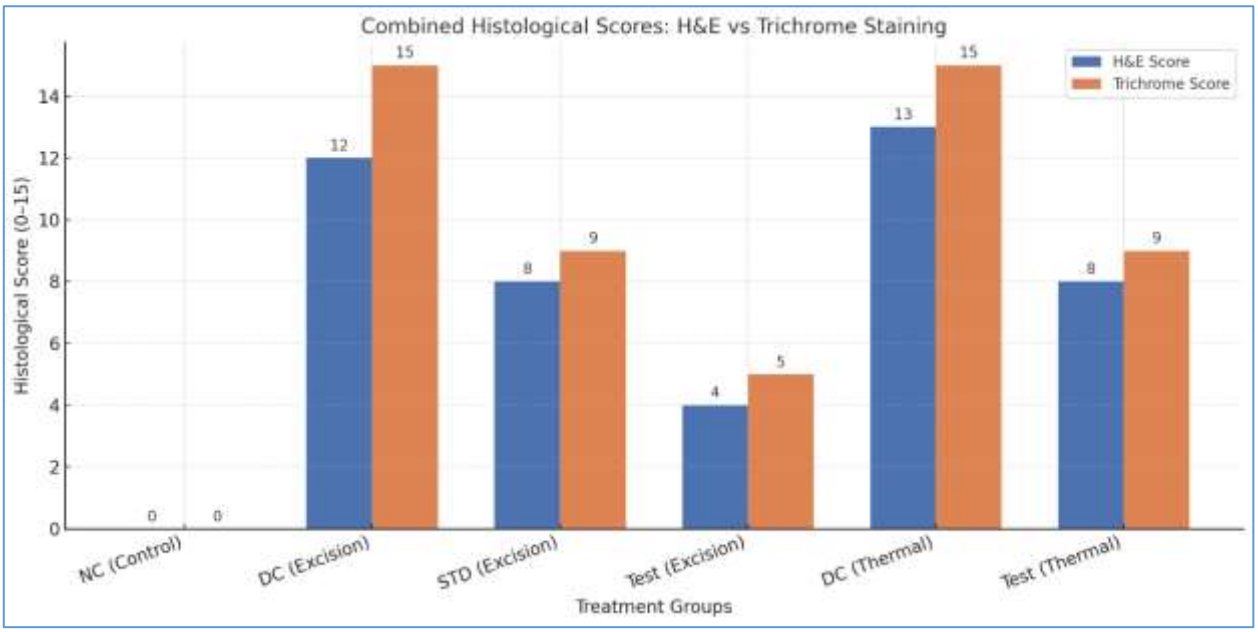


Figure 7: Histological Scores for different groups