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Evaluation of Wound Healing Activity of Mezhugu Kalimbu in Wistar Albino Rats

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ABSTRACT

Background: Chronic wounds continue to pose a serious healthcare challenge, and while Siddha medicine traditionally recommends *Mezhugu Kalimbu* for wound care, scientific validation of its healing properties is scarce. This study was designed to evaluate the wound-healing potential of *Mezhugu Kalimbu* and compare its efficacy with its individual components (*Mezhugu* and coconut oil), standard treatment (povidone iodine), and untreated control.

Materials and Methods: Thirty Wistar albino rats were divided into five groups: untreated, standard (povidone iodine), coconut oil, *Mezhugu*, and *Mezhugu Kalimbu*. Full-thickness excision wounds were created, and topical applications were administered every 48 hours. Wound contraction rate and unit healing time were recorded and analyzed using SPSS.

Results and Discussion: *Mezhugu Kalimbu* group showed significantly greater wound contraction compared to other groups ($p < 0.05$). Complete wound closure was achieved by Day 12, with a 100% mean contraction rate and the shortest mean healing time (8.32 days). The superior healing effect is attributed to the synergistic actions of Mezhugu with moisture-retentive and antibacterial properties and coconut oil, which contributes anti-inflammatory and collagen-promoting effects.

Conclusion: *Mezhugu Kalimbu* demonstrated significantly enhanced wound-healing activity compared to its individual components and the standard treatment, highlighting its potential as an effective, accessible, and affordable wound care formulation within Siddha medicine.

Keywords: Siddha Medicine, *Mezhugu Kalimbu*, Wistar albino rats, wound healing

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INTRODUCTION

Wound healing is a complex biological process involving overlapping phases: hemostasis, inflammation, proliferation and remodeling, each contributing to tissue repair and recovery.[1,2,3] Traditional medical systems, including Siddha Medicine, have long utilized natural preparations for wound care. Siddha Medicine classifies wounds based on their causes, doshic influences and symptoms. Traumatic wounds arise from external injuries such as cuts, burns, and insect or animal bites. Wounds are further categorized according to the tridoshas-*Vata* (*Vazhi Punn*), *Pita* (*Azhal Punn*) and *Kapha* (*Iya Punn*)-each exhibiting distinct characteristics. *Vazhi Punn* is associated with dryness, aching pain and black or pale discoloration; *Azhal Punn* manifests with redness, burning sensations and yellowish or blood-like discharge; while *Iya Punn* presents with swelling, itching and sticky discharges. Mixed dosha wounds, such as *Vazhi Azhal* or *Mukkuttra Punn*, display combined symptoms. Additionally, wounds are classified based on signs and symptoms into infected (*Dushta Viranam*) and clean (Adushta Viranam) types, with the former showing delayed healing, foul-smelling discharge and tissue destruction, while the latter exhibits better healing potential.[4] Traditional medicine provides effective and accessible solutions for wound management, often being more affordable and widely used in communities with limited healthcare

resources. In Siddha medicine, *Mezhugu Kalimbu* a formulation of *Mezhugu* and coconut oil is valued for its antimicrobial, anti-inflammatory, and tissue-regenerative properties, which may support faster and improved wound healing.[3,5,6] Despite its longstanding use, there is limited scientific evidence validating its effectiveness, creating a gap between traditional practice and modern medical knowledge. Wistar albino rats serve as a reliable animal model to evaluate the efficacy of wound-healing agents under controlled experimental conditions, providing insight into their biological effects.[7] Conventional wound treatments, such as antibiotics, synthetic dressings, and surgical procedures, are often expensive, less accessible in rural or resource-limited settings, and may cause side effects.[1] These challenges highlight the need for safe, affordable, and effective alternatives. This study is designed to investigate the wound-healing potential of *Mezhugu Kalimbu* in Wistar albino rats by assessing wound contraction, healing time, and tissue regeneration.

MATERIALS AND METHODS

Study Design: The study was conducted as a controlled laboratory experimental study to evaluate the wound-healing effects of *Mezhugu Kalimbu* in Wistar albino rats. Multiple experimental groups were included to compare the effects of *Mezhugu Kalimbu* with its individual components *Mezhugu* and coconut oil and a standard treatment (povidone iodine), under carefully controlled laboratory conditions.

Study Animal: Wistar albino rats

Study Duration: The experimental study was conducted over six months, from December 2025 to May 2025, allowing adequate time for wound creation, treatment application, and monitoring of healing.

Selection of the Material: *Mezhugu* and Coconut oil, were selected through quotations of general character of the *Mezhugu* and Coconut oil from the book, Gunapadam (dhathu jeeva vaguppu) by *Dr.R. Thiyagarajan*, L.I.M

Collection of Materials: *Mezhugu* was obtained from Paranjothi Ayurvedic Pharmacy, Trincomalee, Sri Lanka

Authentication of Material: *Mezhugu* and Coconut oil were authenticated by the Department of Gunapadam, Faculty of Siddha Medicine, Trincomalee Campus, and Eastern University, Sri Lanka.

Purification of Material: According to *Siddha Marunthu Muraiyiya*l by *Dr.Pon.Ramanathan Mezhugu* is purified by double boiling method which should be melted and allowed to settle. Then filtered to get the purified wax. Coconut oil was prepared, filtered and obtained.

Animal Model: Either sex of thirty healthy Wistar albino rats weighing 150-200g was obtained from Medical Research Institute (MRI) animal house for experimental study.

Preparation of *Mezhugu Kalimbu*: Equal amount of *Mezhugu* and coconut oil were taken and melted then filtered8 and allowed to settle.

Method of getting pure Coconut oil: Coconuts were purchased and the exocarp and mesocarp were removed. And the endocarp (meat) was then blended using a blender.After blending, the coconut particles (chaff) were sieved and squeezed to extract the coconut milk. This was heated to evaporate the water content, leaving the oil, which settled on top of the formed coconut cake.[8]

Adaptation of Animals: Thirty healthy Wistar albino rats, of either sex, were housed in an air-conditioned, well-ventilated room. The animals were fed standard laboratory pellets three times a day and provided with water. Cages for each group were cleaned once every three days throughout the study period to maintain hygienic conditions and minimize stress.

Grouping of Animals: The thirty rats were randomly divided into five groups, with six animals in each group:

Group 1 (Control): Left untreated.

Group 2 (Standard): Treated with the standard wound-healing ointment, povidone iodine.

Group 3 (Test 1): Treated with coconut oil.

Group 4 (Test 2): Treated with *Mezhugu*.

Group 5 (Test 3): Treated with *Mezhugu Kalimbu*.

Excision Wound Model: The surgical interventions carried out under the sterile conditions using ketamine anesthesia (30mg/kg) inject intraperitoneally. Hairs were removed from the dorsal thoracic region of the rats. A circular wound of approximately 120 mm2 marked on the back of the rat by a standard ring. Full thickness of the marked skin was cut carefully. Then Animals kept in separate cages.[10]

Treatment Procedures: Wound dressing for all experimental groups was performed every 48 hours. The assigned treatment was applied topically in a standardized amount of 0.5 g per wound for ointments (*Mezhugu, Mezhugu Kalimbu*, and povidone iodine) and 0.5 ml per wound in the case of coconut oil. Care was taken to cover the entire wound surface evenly without causing additional trauma. The treatments were continued until complete wound closure was observed in all groups.

Assessment: Data was collected once in 2 days from each group and record clearly.

Clinical Evaluation

Wound contraction rate: Wound contraction rate measured by planimetric measurement of wound area on alternate days of post wounding. This done by tracing the wound on a transparent butter paper and then transferred to 1 mm2 graph sheets. Reduction in the wound area expressed as percentage of the original wound size

% wound contraction on day X = [(area on day 0 − open area on day X)/area on day 0] × 100.

Unit Healing Time: Wound healing assessed by UHT (Unit Healing Time) and scoring of signs and symptoms. The UHT means number of days required for healing of per sq. cm area of wound. UHT calculated by the following formula: UHT = TDRH / IAW sq.cm.2

Where, TDRH = Total number of Days Required for Healing and IAW = Initial Area of Wound in square centimeter.

Bates-Jensen Wound Assessment Test: It is an objective measure used to assess wound status and track healing in control, standard and test group.

Significance of the Study: This study will contribute to the scientific validation of traditional Siddha Medicine by investigating the efficacy of *Mezhugu Kalimbu* in wound healing. Positive results could lead to the development of new therapeutic options for wound care.

RESULTS AND DISCUSSIONS

Table 1: The ANOVA One-way statistic for circumference of the wound

ANOVA						
		Sum of Squares	Df	Mean Square	F	Sig.
Day 00	Between Groups	1.867	4	.467	.598	.667
	Within Groups	19.500	25	.780		
	Total	21.367	29			
Day 02	Between Groups	.800	4	.200	.750	.567
	Within Groups	6.667	25	.267		
	Total	7.467	29			
Day 04	Between Groups	2.133	4	.533	.792	.541
	Within Groups	16.833	25	.673		
	Total	18.967	29			
Day 06	Between Groups	3.200	4	.800	1.111	.373
	Within Groups	18.000	25	.720		
	Total	21.200	29			
Day 08	Between Groups	1716.467	4	429.117	143.039	.000
	Within Groups	75.000	25	3.000		
	Total	1791.467	29			
Day 10	Between Groups	4068.800	4	1017.200	807.302	.000
	Within Groups	31.500	25	1.260		
	Total	4100.300	29			
Day 12	Between Groups	8960.467	4	2240.117	1877.193	.000
	Within Groups	29.833	25	1.193		
	Total	8990.300	29			
Day 14	Between Groups	1704.867	4	426.217	560.811	.000
	Within Groups	19.000	25	.760		
	Total	1723.867	29			

(Results were statistically analyzed using SPSS.)

The ANOVA analysis (Table 1) showed no significant differences among the five groups during the early phase (Day 0–Day 6), confirming baseline homogeneity. From Day 8 onward, statistically significant differences emerged ($p < 0.001$), with F-values increasing sharply and reaching a peak on Day 12, indicating the strongest treatment effect at this stage. Although the F-value declined slightly by Day 14, it remained highly significant, demonstrating a sustained impact of the intervention.

Table 2: Post Hoc (Tukey's HSD) for Contraction Rates among groups over Days.

Multiple Comparisons							
Tukey HSD							
Dependent Variable	**(I) Rats**	**(J) Rats**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Day 08	Untreated	Standard	9.167*	1.000	.000	6.23	12.10
		Coconut oil	9.833*	1.000	.000	6.90	12.77
		Mezhugu	1.333	1.000	.674	-1.60	4.27
		Mezhugu Kalimbu	21.167*	1.000	.000	18.23	24.10
	Standard	Untreated	-9.167*	1.000	.000	-12.10	-6.23
		Coconut oil	.667	1.000	.962	-2.27	3.60
		Mezhugu	-7.833*	1.000	.000	-10.77	-4.90
		Mezhugu Kalimbu	12.000*	1.000	.000	9.06	14.94
	Coconut oil	Untreated	-9.833*	1.000	.000	-12.77	-6.90
		Standard	-.667	1.000	.962	-3.60	2.27
		Mezhugu	-8.500*	1.000	.000	-11.44	-5.56
		Mezhugu Kalimbu	11.333*	1.000	.000	8.40	14.27
	Mezhugu	Untreated	-1.333	1.000	.674	-4.27	1.60
		Standard	7.833*	1.000	.000	4.90	10.77
		Coconut oil	8.500*	1.000	.000	5.56	11.44
		Mezhugu Kalimbu	19.833*	1.000	.000	16.90	22.77
	Mezhugu Kalimbu	Untreated	-21.167*	1.000	.000	-24.10	-18.23
		Standard	-12.000*	1.000	.000	-14.94	-9.06
		Coconut oil	-11.333*	1.000	.000	-14.27	-8.40
		Mezhugu	-19.833*	1.000	.000	-22.77	-16.90

(Results were statistically analyzed using SPSS.)

The post-hoc multiple comparisons test (Table 2) showed, From Day 8 onwards, treatment effects began to emerge, with larger mean differences observed between the untreated group and those receiving *Mezhugu* or *Mezhugu Kalimbu*. These findings indicate a delayed but progressively stronger treatment effect, with Mezhugu based preparations demonstrating superior wound-healing outcomes compared to untreated and standard groups in the later stages of observation.

From Day 8th, the *Mezhugu Kalimbu* group shows a marked acceleration in contraction rate, surpassing all other groups and reaching 100% by Day 12th, indicating complete wound closure. Interestingly, Coconut oil outperforms the Standard treatment, achieving a higher wound contraction rate more rapidly and nearing complete healing just before Day 14th. This suggests that Coconut oil has a more pronounced effect on promoting wound healing than the Standard treatment. The Standard group follows closely behind but lags slightly in effectiveness.

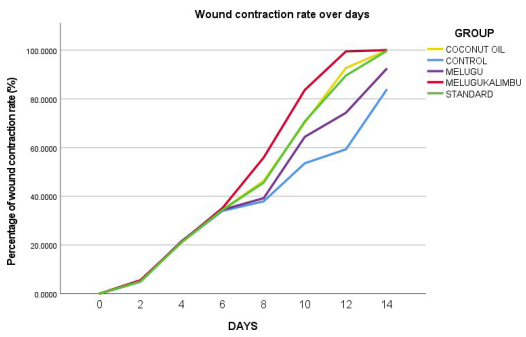


Fig 1: Wound Contraction Rate

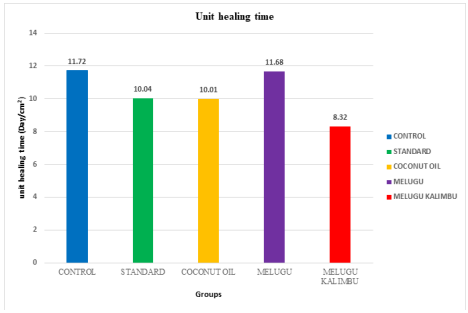
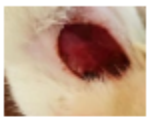
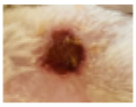
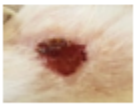
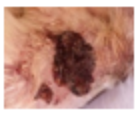


Fig 2: Unit Healing Time over days

The Mezhugu Kalimbu group demonstrated the lowest healing time at 8.32 units, significantly outperforming all other groups. This suggests that Mezhugu Kalimbu has superior wound-healing properties compared to the other treatments and the Control group, making it a potentially highly effective intervention. The results highlight the variability in healing efficacy among the treatments, with Mezhugu Kalimbu standing out as the most potent.

Table 3 illustrates the wound size reduction (cm^2) over time for the Control group, Povidone Iodine, Coconut Oil, Mezhugu and Mezhugu Kalimbu. The images presents the progressive decrease in wound area from Day 0 to Day 14, highlighting the effectiveness of each treatment in promoting wound healing. The Control group exhibited the slowest reduction in wound size, retaining a larger wound area throughout the study, indicating delayed healing. Povidone Iodine facilitated moderate wound contraction, showing better results than the Control group but slower than the test drugs. Coconut Oil and Mezhugu Kalimbu demonstrated a more significant reduction in wound size, supporting their effectiveness in accelerating tissue repair. So the wound size reduction is superior for Mezhugu Kalimbu, Coconut oil, Standard, Mezhugu and Control respectively.

Table 3: Photographic Comparison of Wound Healing Progression across Groups

	Day 00	Day 02	Day 04	Day 06	Day 08	Day 10	Day 12	Day 14
Mezhugu kalimbu								
Coconut oil								
Povidone								
Mezhugu								
Control								

Mezhugu and Its Therapeutic Role

Mezhugu, a natural secretion of honeybees, has been extensively used in traditional medicine for its protective, antimicrobial and emollient properties. It acts as an occlusive agent that prevents Transepidermal water loss while allowing the skin to breathe. Studies have confirmed *Mezhugu's* antibacterial activity, particularly against Staphylococcus aureus, a common wound pathogen.[10,11] The present findings Moreover, *Mezhugu* contains esters and fatty acids that contribute to anti-inflammatory and moisturizing effects, both crucial in the wound healing process. The protective barrier formed by *Mezhugu* maintains an optimal moist environment, promoting fibroblast proliferation and epithelial cell migration. A study indicated that *Mezhugu* formulations enhanced granulation tissue formation and reduced oxidative stress, which aligns with our observations of improved wound closure and reduced inflammatory infiltrates.[12]

In addition to the observed effects of *Mezhugu* and coconut oil, it is important to consider the potential contribution of honey in wound healing, particularly when used in unrefined forms where traces of honey may remain in *Mezhugu*. The sweet taste of honey, attributed to its high sugar content (primarily glucose and fructose), plays a significant biological role in wound healing. The hyperosmolar nature of honey due to its sugar concentration draws fluid from the wound and surrounding tissues, creating an environment that inhibits microbial growth. This osmotic effect also helps to reduce edema and supports the natural cleansing of the wound bed.

Coconut oil and Its Biological Actions

Coconut oil, particularly virgin coconut oil (VCO), is rich in medium-chain triglycerides, notably lauric acid, which possesses broad-spectrum antimicrobial properties. VCO significantly improved antioxidant enzyme activity in wounds and increased collagen cross-linking, which accelerates tissue repair.[13] The

findings mirror this, with the coconut oil-treated group showing faster wound contraction and better collagen deposition under histological evaluation.

The anti-inflammatory effect of coconut oil has also been reported that topical application reduced edema and neutrophil infiltration in rat models.[14] This is consistent with the reduced inflammatory signs observed in our study, suggesting that coconut oil not only prevents microbial colonization but also modulates the inflammatory phase of healing.

Synergistic Effects of Mezhugu and Coconut Oil

Interestingly, the study observed that the combination of *Mezhugu* and coconut oil yielded superior wound healing compared to their individual use. This synergistic effect is likely due to the complementary actions of both components- *Mezhugu* forming a protective barrier and coconut oil providing antimicrobial, antioxidant and anti-inflammatory benefits.

Previous research supported this idea, reporting enhanced skin permeability and drug retention when natural lipids like Mezhugu were combined with oils.[15] Additionally, a study found that formulations containing both Mezhugu and plant oils facilitated re-epithelialization and improved cosmetic outcomes in wound healing, which is also evident in our results.[16]

Taken together, our study reinforces existing literature that identifies both *Mezhugu* and coconut oil as potent wound healing agents. Their individual contributions to antimicrobial defense, inflammation control and tissue regeneration are well documented. More importantly, our research adds to the evidence supporting the enhanced efficacy of combining these natural substances for wound care applications.

Role of sweet taste of *Mezhugu* in healing wound

Siddha Medicine offer profound insights into the dietary and pharmacological factors that influence wound recovery. One such important principle is the therapeutic value of Madhura suvai (sweet taste).

In Siddha medicine, as emphasized by *Uthamarayan, Mathura suvai* (sweet taste) is believed to strengthen the seven body constituents (udal thathukkal). This holistic nourishment ensures proper tissue regeneration and repair. Sweet taste also promotes the growth of hair, sharpens the longevity and function of the five sense organs and pacifies aggravated Vatha and Pitha doshas, which are commonly involved in wound pathogenesis.

According to recent reviews, *Madhura suvai* is composed predominantly of the earth and water elements making it inherently nourishing and tissue-binding in nature. It is especially recommended during the proliferative and remodeling phases of wound healing, where it helps in granulation tissue formation, strengthening of new tissue and promotion of body constituents.

A narrative highlights that foods with *Madhura suwai* possess cold potency (*Seetha Viriyam*) and sweet post-digestive effect (*Madhura Vipakam*), making them suitable for controlling inflammation and enhancing regenerative processes.[17] These attributes aid in maintaining doshic balance, particularly in reducing Vatha and Pitta vitiation, which are known to delay wound healing.

The formulation *Mezhugu Kalimbu*, containing *Mezhugu* and coconut oil both predominantly sweet in taste exemplifies the practical application of this principle. These ingredients provide unctuousness (*Snigdha Guna*), act as barriers to infection, retain moisture and enhance cellular proliferation at the wound site. Their systemic and local effects align with the classical understanding of how sweet taste contributes to tissue repair, strength and restoration.

Thus, the integration of *Mathura suvai* into wound healing regimens is not only supported by traditional doctrines but also rationalized through modern research. Recognizing and applying this ancient wisdom in clinical practice provides a natural, safe and effective approach to accelerate healing and improve outcomes in chronic and acute wounds.

The results of the present study indicate that *Mezhugu Kalimbu*, a formulation containing *Mezhugu* and Coconut oil, significantly accelerated wound healing in Wistar Albino Rats compared to the control group. Parameters such as wound contraction rate and epithelialization period demonstrated marked improvement in the treated group. The observed effects may be attributed to the synergistic actions of the traditional *Mezhugu* preparation and the known anti-inflammatory, antimicrobial and moisturizing properties of coconut oil.

CONCLUSION

This study concluded that *Mezhugu Kalimbu* significantly enhances wound healing compared to the application of coconut oil, *Mezhugu* alone, standard treatment (povidone iodine), and control groups. The formulation demonstrated a substantial reduction in wound size, accelerated re-epithelialization, reduced inflammation, and faster overall healing, with statistically significant outcomes ($p < 0.05$). The observed effects can be attributed to the synergistic action of *Mezhugu's* antimicrobial and barrier-forming properties along with the anti-inflammatory and collagen-stimulating effects of coconut oil. Owing to their natural origin, cost-effectiveness, and safety profile, this combination offers a promising and accessible alternative for effective wound care management.

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