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Research

Topical application of Gula Tunub (local honey) from Lanao Del Sur, Philippines to Albino Mice (*Mus Musculus*) as a treatment of open wounds

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	Abstract
Published on: 29 Jun 2024	Background: This experimental study aimed to evaluate the wound healing potential of Gula Tunub, a unique honey variety from Lanao del Sur, Philippines, in Albino mice.
Published by: DrSriram Publications	Methods: Conducted from December 19, 2023, to December 29, 2023, at Mindanao State University, Marawi City, Lanao del Sur, Southern Philippines, the research involved 45 Albino mice aged 3 to 6 months, distributed into three groups: T1 (100% Gula Tunub), T2 (Mixed Gula Tunub), and T3 (100% Normal Saline Solution). The study employed a controlled experiment with clinical observation methods, including wound excision model, treatment administration, and wound measurement. Data analysis involved One-Factor ANOVA and multiple comparison tests such as Tukey's HSD Test, Scheffé Test, and Bonferroni-Holm's Test.
2024 All rights reserved.  Creative Commons Attribution 4.0 International License.	Results: Findings indicated significant wound area reduction in the T1 group (100% Gula Tunub) over a 7-day period, suggesting its potential effectiveness in promoting wound healing. The T2 group (Mixed Gula Tunub) also showed promising results, with most mice achieving near-complete or complete healing by Days 6 to 7. The control group (T3) treated with Normal Saline Solution exhibited minimal progress in wound area reduction. Both Gula Tunub treatments demonstrated accelerated wound healing progress compared to the control. Conclusion: The study suggests that Gula Tunub honey, especially in 100% and mixed concentrations, has potential as a wound healing treatment in Albino mice, surpassing the effects of Normal Saline Solution. Further research should delve into the mechanisms of Gula Tunub honey's healing properties for potential clinical applications in wound care. Ethical guidelines for animal research were followed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Keywords: Gula Tunub, honey, Albino Mice, wound healing, experimental, Lanao del Sur, Philippines.

INTRODUCTION

Open wounds are a common medical condition that, if left untreated, can develop a number of complications. With the advent of cutting-edge treatments like growth factors, skin substitutes, and negative pressure wound therapy, the treatment of wounds has changed over time. However, not all populations can always afford these cutting-edge treatments, and they are frequently expensive. Owing to their accessibility, low cost, and negligible side effects, natural products have attracted increasing attention as potential treatments for wound healing in recent years.¹

Traditional remedies, including honey, have been used for centuries to promote wound healing. Honey has therapeutic properties such as antimicrobial and anti-inflammatory effects that make it a promising candidate for wound management². Research has shown its potential in speeding up the healing of wounds and promoting tissue regeneration in animal models. Certain varieties of local honey, like Gula Tunub from the Lanao del Sur region of the Philippines, are documented to have unique therapeutic properties.³

The research gap in the topical application of Gula Tunub (local honey) from Lanao del Sur as a treatment for open wounds in Albino Mice (*Mus musculus*) lies in the limited exploration of this specific honey type's potential for wound healing. While numerous studies have investigated the wound healing properties of various honey types, the unique composition and therapeutic potential of Gula Tunub from the Lanao del Sur region have not been extensively studied. Additionally, although topical honey applications have gained attention, most research focuses on systemic honey administration.⁴⁻⁶ This study aims to address this gap by evaluating the efficacy of Gula Tunub in promoting wound healing when applied topically to Albino Mice, providing valuable insights into the potential of this local honey for wound management.

METHODS AND MATERIALS

Research Design

The study examined the impact of Gula Tunub honey on wound healing in Albino Mice using a Completely Randomized Design (CRD). The Completely Randomized Design (CRD) is a widely utilized experimental design in various research fields due to its straightforward nature and ease of implementation (Lawal, 2014). In this specific study, the CRD was chosen for its simplicity and the ability to investigate the effects of different honey treatments on wound healing in Albino Mice without the need for complex designs.

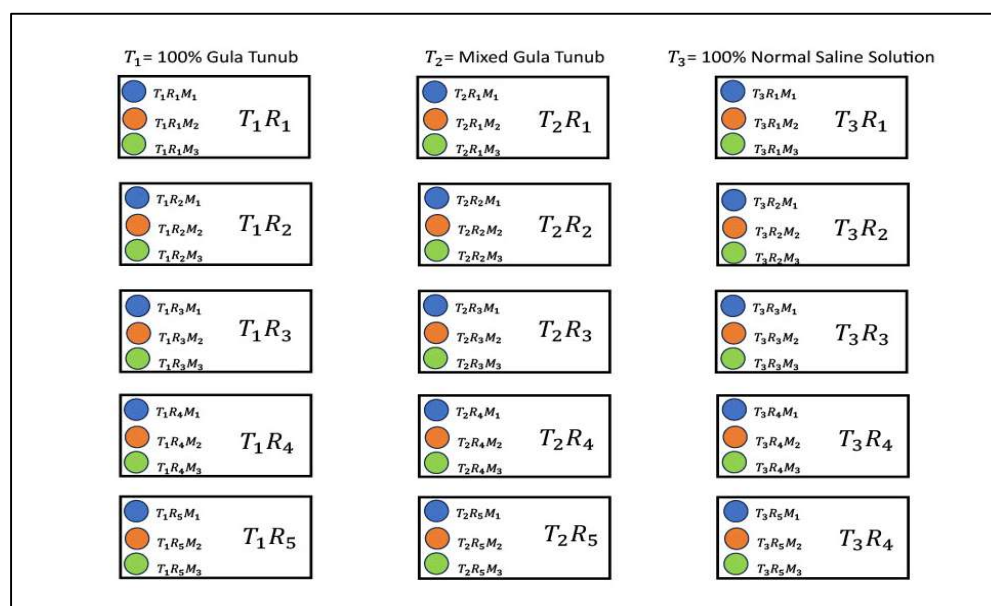
Research Locale and Setting

The research locale was strategically chosen to facilitate the experiment on the 45 Albino Mice. To ensure proper care and monitoring, each mouse was housed in a separate transparent cage measuring 0.2667 meters long, 0.1905 meters wide, and 0.1778 meters high (10.4 square meters area). The study began on December 19, 2023, concluding on December 29, with a preceding acclimatization period of ten days for the mice to adjust to their surroundings before the actual procedure started.

Research Subjects

The study involved a total of forty-five experimental units (mice) with five replications, each containing three experimental units. Albino Mice aged 3 to 6 months old weighing 13-22 grams were selected for the study due to their genetic, biological, and behavioural similarities to humans.

The experimental study used simple random sampling to allocate Albino Mice to the three treatment groups - T1 (100% Gula Tunub honey), T2, and T3 (100% Normal Saline Solution). Each group had five replications, with each replication containing three mice, resulting in 15 mice in each treatment group. The dice method was employed for assigning the mice to the treatment groups, ensuring a fair and unbiased distribution among them.



Note: T = Concentration of Gula Tunub; R = Replication; M = Mic

Fig 1: Random Distribution of Experimental Units

Data Gathering Procedures

The data gathering procedures for the experimental study on Gula Tunub honey's topical application in wound healing among Albino Mice were meticulously planned. The study began on December 19, 2023, concluding on December 29, 2024, with a preceding acclimatization period of ten days for the mice to adjust to their surroundings before the actual procedure started. Initial steps involved securing ethical approvals and permissions.

Clinical observation served as the primary data collection method, with close monitoring of wound progress and adverse effects. Mice were identified with non-toxic markers and sedated for procedures. Sterile environments were maintained for incisions, with careful shaving and positioning. Treatments, including pure honey, honey-saline mixture, and saline, were applied daily for seven days. Mice were housed individually with proper care. Wound diameter was measured using a vernier caliper, and area calculated for analysis. Data were analyzed using One-Factor ANOVA and multiple comparison tests, allowing meaningful conclusions on Gula Tunub honey's efficacy in wound healing. Overall, the study demonstrated a thorough approach, ensuring reliable findings on the honey's potential benefits.

Methods of Data Analysis

Data were analyzed using One-Factor ANOVA and multiple comparison tests, including Tukey's HSD Test, Scheffé Test, and Bonferroni-Holm's Test. The study aimed to determine the progress of wound area reduction among mice treated with different concentrations of Gula Tunub honey over a 7-day period and compare the mean reductions.

Ethical Considerations

The experimental study on the topical application of Gula Tunub honey for wound healing in Albino Mice rigorously adhered to the National Institutes of Health's (2011) Guide for the Care and Use of Laboratory Animals, ensuring ethical standards were met. The study obtained institutional approval from an Animal Care and Use Committee (IACUC) before initiation, implemented measures to minimize the number of mice used through proper experimental design, selected genetically consistent Albino Mice, utilized anesthesia and analgesia for invasive procedures, monitored the mice's health throughout the study, and conducted humane euthanasia at the study's conclusion or when deemed necessary by the IACUC.

RESULTS

Wound Area Reduction

Table 1: Mean Percentage of Wound Area Reduction Daily in All Treatments within 7 Days

Treatment	Percentage of wound closure daily within 7 days						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
T ₁ (100% Gula Tunub)	0%	48.66%	80.37%	92.26%	96.94%	98.70%	99.93%
T ₂ (Mixed Gula Tunub)	0%	54.38%	80.06%	86.97%	93.42%	97.95%	99.41%
T ₃ (100% NSS)	0%	36.52%	67.45%	79.01%	89.49%	96.43%	98.85%

Note: NSS = Normal Saline Solution

In the experimental study, the group treated with 100% Gula Tunub honey (T1) displayed a notable decrease in wound area over 7 days, with the majority of mice achieving complete epithelialization by Day 7. Similarly, the group treated with a 50% Gula Tunub mixture (T2) also showed significant wound area reduction, with most mice nearing complete healing by Day 7. In contrast, the control group (T3) treated with normal saline solution exhibited slower progress in wound healing, with only a few mice achieving complete epithelialization within the observation period. These findings underscore the potential benefits of using Gula Tunub honey for wound healing compared to normal saline solution.

Table 1 illustrates the daily progress of wound area reduction in albino mice treated with varying concentrations of Gula Tunub honey over a 7-day period. Analysis of the data reveals that both Gula Tunub honey treatments (T1 and T2) significantly accelerated wound healing compared to normal saline solution treatment (T3). Initially, the mixed Gula Tunub honey treatment (T2) showed slightly higher wound closure percentages than the 100% Gula Tunub honey treatment (T1) on Day 1, but T1 exhibited a slightly greater rate of closure starting from Day 2.

Significant Daily Variations in the Reduction of the Wound Area Between Treatments

The subsequent tables employ a One-Way Analysis of Variance (ANOVA) to determine the statistical significance of the observed wound area reduction in albino mice treated with different concentrations of Gula Tunub honey for each day. In order to establish a significant difference, the F value must exceed the F critical value ($F > 3.23$) at a 5% significance level ($p \leq 0.05$). This method allows for a comprehensive evaluation of the treatment groups' efficacy in promoting wound healing.

Table 2: Summary of Results in the Daily Variations of the Reduction of the Wound Area Between Treatments

Wound Area Reduction From Day 1 to Day 7			
Day	F	p-Value	Interpretation
Day 1		1.0000 <i>n.s</i>	Not Significant
Day 2	4.2758	0.0204*	Significant
Day 3	5.1538	0.0100*	Significant
Day 4	6.2311	0.0043*	Significant
Day 5	6.0588	0.0049*	Significant
Day 6	1.5611	0.2218 <i>n.s</i>	Not Significant
Day 7	1.3914	0.2600 <i>n.s</i>	Not Significant

**Significant at $p \leq 0.05$; n.s = not significant*

The results of the daily variations in wound area reduction in Albino mice treated with different concentrations of Gula Tunub honey are summarized in Table 2. Statistically significant differences were observed in wound area reduction on Days 2 to 5, indicating a more pronounced effect of Gula Tunub honey during these days. While Day 1 showed no significant difference among treatment groups, suggesting a delayed impact of honey on wound healing, subsequent days demonstrated significant variations in wound area reduction between treatment concentrations. This aligns with the reported antimicrobial, anti-inflammatory, and wound healing properties of honey. However, no significant differences were noted on Days 6 and 7, possibly indicating a plateau in the healing process. Further research is warranted to determine optimal concentrations and application durations of Gula Tunub honey for maximal wound healing benefits, considering factors like wound severity and overall health status.

Table 3: Summary of Results in the Daily Variations of the Reduction of the Wound Area Between Treatments Using One-Way ANOVA

Wound Area Reduction From Day 1 to Day 7					
Sources of Variation	SS	Df	MS	F	p-Value
Between Treatments	2.2514	2	1.1257	5.8064	0.0059
Error	8.1427	42	0.1939		
Total	10.3942	44			

Note: SS = Sums of Squares, df = Degrees of Freedom, MS = Means Squares $F = F\text{-ratio}$, $p \leq 0.05$

Table 3 presents the One-Way ANOVA summary table for wound area reduction from Day 1 to Day 7, dividing results by 7 to represent sample means for each experimental unit. The analysis indicates a significant difference among treatments in reducing wound area over 7 days. The F-value of 5.8064, associated with a p-value of 0.0059 (<0.05), suggests at least one treatment significantly impacts wound healing compared to others. This statistical insight underscores the effectiveness of treatments in promoting wound healing and guides further research into optimal treatment strategies.

Multiple Comparison Tests Among Treatments

Given the outcomes in the daily variations in the reduction of the wound areas between treatments using One-Way ANOVA, it is crucial to conduct multiple comparison tests among the treatments using post-hoc tests. These tests, such as Tukey HSD, Scheffé, Bonferroni, and Holm multiple comparison tests, will help identify which specific pairs of treatments exhibit significant differences in their ability to reduce wound area. The importance of these post-hoc tests lies in their ability to provide a more detailed understanding of the treatments' effectiveness. By determining which treatments are significantly different from each other, researchers can gain valuable insights into the factors contributing to wound healing and potentially develop more efficient therapies in the future.

Table 4: Tukey's Honest Significance Difference Test

Treatments Pair	Tukey HSD Q Statistic	Tukey HSD p -Value
T_1 vs. T_2	0.4222	0.8999947 ^{ns}
T_1 vs. T_3	4.3687	0.0097344**
T_2 vs. T_3	3.9465	0.0210603*

Note: T_1 = 100% Gula Tunub; T_2 = Mixed Gula Tunub; T_3 = 100% Normal Saline Solution

*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$; $n.s$ = not significant

In daily variations, the One-Way ANOVA showed differences in wound area reduction between treatments. It is crucial to conduct post-hoc tests such as Tukey HSD, Scheffé, Bonferroni, and Holm multiple comparison tests to identify specific treatment pairs with significant differences in reducing wound area. These tests provide detailed understanding of treatment effectiveness and insights into factors contributing to wound healing for potential development of more efficient therapies.

Table 4 summarizes the results of the Tukey's Honest Significance Difference Test, indicating significant differences among pairs of treatments (T_1 , T_2 , and T_3) in reducing wound area over a 7-day period. The comparison between T_1 and T_2 shows no significant difference, while both T_1 and T_2 are significantly more effective than treatment T_3 in reducing wound area.

Table 5: Scheffé Significance Difference Test

Treatments Pair	Scheffé T -Statistic	Scheffé p -Value
T_1 vs. T_2	0.2985	0.9564588 ^{ns}
T_1 vs. T_3	3.00891	0.0135747*
T_2 vs. T_3	2.7906	0.0281024*

Note: T_1 = 100% Gula Tunub; T_2 = Mixed Gula Tunub; T_3 = 100% Normal Saline Solution

*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$; $n.s$ = not significant

Table 5 presents the results of the Scheffé Test Significance Difference, another post-hoc test applied to determine significant differences among pairs of treatments (T_1 , T_2 , and T_3) in decreasing wound area over seven days. The findings indicate that there is no substantial distinction between treatments T_1 and T_2 in terms of wound area reduction ($p = 0.956$). However, comparisons between T_1 vs. T_3 ($p = 0.013$) and T_2 vs. T_3 ($p = 0.028$) show significant differences at a significance level of $p \leq 0.05$, suggesting that Gula Tunub treatments have distinct effects on wound area compared to Normal Saline Solution.

Table 6: Bonferroni-Holm's Significance Difference Test

Treatments Pair	Bonferroni-Holm <i>T</i> -Statistic	Bonferroni <i>p</i> -Value	Holm <i>p</i> -Value
T ₁ vs. T ₂	0.2985	2.3002761 ^{ns}	0.7667587 ^{ns}
T ₁ vs. T ₃	3.0891	0.0106598*	0.0106598*
T ₂ vs. T ₃	2.7906	0.0236410*	0.0157607*

Note: T₁ = 100% Gula Tunub; T₂ = Mixed Gula Tunub; T₃ = 100% Normal Saline Solution

*Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$; *n.s.* = not significant

The results of the Bonferroni-Holm test indicate that there are significant differences between T1 and T3, as well as between T2 and T3. However, there was no significant difference between T1 and T2, implying that these two treatment groups may have similar effects. In summary, the outcomes of the Bonferroni-Significance Holm's Difference Test demonstrate that both the 100% Gula Tunub treatment, T1 and the mixed Gula Tunub treatment, T2 show significant differences in their ability to reduce wound area compared to the 100% Normal Saline Solution treatment (T3).

DISCUSSION

The major findings suggest that the topical application of 100% pure Gula Tunub honey significantly promoted wound healing in Albino Mice. This observed reduction in wound area in the experimental group T1 can be attributed to Gula Tunub honey's reported wound-healing properties, including antioxidants, antimicrobial agents, and anti-inflammatory agents, sourced from Lanao del Sur, Philippines⁴ (Ahmed & Othman, 2013). These components contribute to tissue regeneration, inflammation reduction, and infection prevention, expediting the healing process⁷. This outcome aligns with previous studies demonstrating honey's efficacy in wound healing, such as those by^{8,9} Pleeing et al. (2022) and Putri et al. (2021), which showed faster healing rates and reduced wound areas in treated wounds compared to controls.¹⁰

Moreover, the observed reduction in wound area in both T1 and T2 groups underscores Gula Tunub honey's therapeutic properties. Honey's antimicrobial, anti-inflammatory, and wound healing properties, attributed to its natural composition, including hydrogen peroxide and various enzymes and bioactive compounds, accelerate wound healing processes.¹¹ Conversely, the lack of substantial improvement in the 100% normal saline solution group can be attributed to its isotonic nature, lacking the therapeutic attributes of honey. This is supported by research highlighting honey's unique healing properties over saline solutions, such as those by^{12,13} Kakakhel et al. (2022) and Justus (2019).

The findings from this experimental study emphasize the importance of using appropriate wound dressings with therapeutic properties to enhance wound healing. The results highlight the potential of Gula Tunub honey as a natural and effective wound healing agent. However, further research is necessary to establish a more comprehensive understanding of the underlying mechanisms and to compare the efficacy of Gula Tunub honey with other types of honey or conventional wound dressings.

The results from multiple comparison tests among treatments further affirm the superiority of Gula Tunub honey treatments in reducing wound area compared to normal saline solution. Studies by¹⁴⁻¹⁶ Chaudhary et al. (2020), Kassem et al. (2023), and Yamani & Fakiha, (2023) consistently demonstrate honey's effectiveness in reducing wound area and promoting faster healing compared to saline solutions. These findings collectively emphasize the potential of Gula Tunub honey as a natural and effective wound healing agent, warranting further research to elucidate optimal concentrations and application durations for maximal benefits. Overall, the study underscores the importance of utilizing wound dressings with therapeutic properties and highlights Gula Tunub honey's promise in wound care applications.

CONCLUSION

The study highlights Gula Tunub honey's potential to expedite wound healing in Albino Mice, with implications for healthcare practices. Utilizing the Health Promotion Model, it elucidates how personal, behavioral, and environmental factors influence the efficacy of Gula Tunub honey in wound healing. Further investigations are warranted to explore its applicability in human wound care and to optimize its usage for maximal effectiveness. The significant effectiveness of 100% pure Gula Tunub honey in promoting wound healing among Albino Mice suggests its viability as a natural remedy for wound management. This finding holds significance for healthcare practitioners, researchers, and the public, underscoring the potential of Gula Tunub honey in enhancing wound care practices.

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Conflict of Interest

The authors declare that they have no competing interests.

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