Green Medical Journal Vol.8 Issue: 1 (April, 2026): 1-13
E-ISSN: 2686-6668
DOI: 10.33096/37dg8p79

Green Medical Journal
Volume 8 Issue 1

Original Article Open Access

The Effect of Trigona Honey as a Therapy for Burns in Rattus Norvegicus

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ABSTRACT

Introduction: Wound healing is an important physiological process to maintain skin integrity after trauma. Contact burns are damage or loss of tissue caused by heat sources. The growing interest in natural therapies has prompted the exploration of trigona honey due to its antioxidant, anti-inflammatory, and antimicrobial properties. Another study discusses trigona honey on an incised wound, but only a few discuss burns. This study evaluates the effectiveness of Trigona spp honey as a therapy for burns in Rattus norvegicus.
Methods: A quasi-experimental with a non-randomized control group pretest-posttest design method. In this study used 20 subjects, there are Rattus norvegicus of the Sprague Dawley strain weighing 100-250 grams and aged 2-3 months, divided into 5 subjects for 4 groups. This study assesses wound size and use one-way ANOVA as the analysis technique.
Result: There is significant wound healing initially in all groups before intervention, average wound area is 3.5 cm. Then treatment was given for 15 days and it was seen that the group with the best wound healing was treatment on group II (Trigona honey 2 g) with an average wound area of 0.42 cm, followed by treatment group I (Trigona honey 1 g) is 0.72 cm, followed by the positive control group (silver sulfadizine 0.5 g) is 0.86 cm and then on negative control group (aquadest) is 1.74 cm. In the one-way ANOVA test, the *P value* (Between Groups) was 0.000 (<5%).
Conclusion: Trigona honey is effective as a therapy for burns in rattus norvegicus.

Keywords: Burns; honey; trigona spp; rattus norvegicus

GREEN MEDICAL JOURNAL
E-ISSN 2686-6668

Article history:
Received: 24 January 2026
Accepted: 20 April 2026
Published: 29 April 2026

Published by:
Faculty of Medicine
Universitas Muslim Indonesia

Address:
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Introduction

Wound healing is an important physiological process to maintain the integrity of the skin after trauma. Functional improvement and anatomical continuity are complex dynamic processes in wound healing.[1] Burns are damage or loss of tissue caused by contact with a heat source such as flames, exposure to hot water, contact with hot objects, electric shock, chemicals and sunburn.[2]

Some plants that are useful in burn therapy include: aloe vera, honey, noni leaves, cherry leaves, mangosteen peel, guava leaves, annona leaves, gotu kola herb, seaweed, betel leaves and banana plants. The compounds contained medicinal plants that are useful in accelerating burn wound closure include flavonoids, tannins, saponins, polyphenols, alkaloids, vitamin B, vitamin C, as well as enzymes and amino acids.[3-5]

Honey has been used by humans for a long time, as a mixture of food and drinks as a sweetener and flavoring agent. Since ancient times, honey has been known, for its nutritional value and therapeutic effects.[6] Based on existing studies, honey has been proven to have antioxidant activity because it contains high levels of flavonoids. Other antioxidants contained in honey are protein and amino acids as well as polyphenol group phytochemicals (quercetin-3, caffeic acid, gallic acid, apigenin, cinnamic acid and catechin). Apart from that, honey has also been proven to contain several enzymes, including glucose oxidase and catalase, as well as several vitamins that are important for the body, such as vitamins A, B complex, C and E as antioxidants.[7-10] The reason for carrying out this study is to contribute to *Thibb Al-Nabawi*, which is sourced from the Al-Qur'an in surah An-Nahl [16] verses 68-69.[11] Trigona honey was administered orally to rodents at doses ranging from 1 to 2 g/kg/day as previously used in clinical trials for increasing antioxidant levels. [12,13]

Methods

Study Design

This study is a quasi-experimental, utilizing a non-randomized control group pretest-posttest design method. In this study were used 20 subjects, consisting of Rattus norvegicus of the Sprague Dawley strain weighing 100-250 grams and aged 2-3 months, divided into 5 subjects across 4 groups. This study assessed wound size and used one-way ANOVA as the technique analysis.[14,15]

One-way ANOVA:

Minimum: $n = 10/k + 1$; n= 10/4 + 1 = 3,5
Maximum: $n = 20/k + 1$; n= 20/4 + 1 = 6

Note: k = number of groups

Study Site

This study was conducted from August to October 2024. The location for testing honey's active substances was at Health Laboratory Center South Sulawesi Province and the intervention was carried out at the Animal Laboratory, Faculty of Medicine, Universitas Muslim Indonesia

Materials

This study used a material included trigona honey, silver sulfadiazine ointment, distilled water and lidocaine prilocaine (5%) cream.

Result

The results of the observations are presented in the following table:

Table 1. The size of the burn wound on before and after intervention

Groups	Wound area (pre-intervention) (cm)			Wound area (post-intervention (cm)		
	Lenght	**Widht**	**Wound area**	**Lenght**	**widht**	**Wound area**
Treatment Group 1 (Trigona Honey 1 g)	2,1	2	4,2	0,98	0,98	0,96
	2,1	1,8	3,78	0,94	0,89	0,84
	1,8	2,1	3,78	0,93	0,51	0,47
	2	1,9	3,8	0,94	0,79	0,74
	1,8	1,8	3,24	1	0,59	0,59
Treatment Group 2 (Trigona Honey 2 g)	1,8	2	3,6	0,97	1,06	1,03
	2	1,6	3,2	0,97	0,23	0,22
	1,8	1,8	3,24	0,57	0,47	0,27
	1,7	1,6	2,72	0,43	0,53	0,23
	1,8	1,7	3,06	0,74	0,53	0,39
Positive control: Silver Sulfadiazine 0,5 g)	1,9	1,8	3,42	0,88	0,83	0,73
	1,9	1,8	3,42	0,93	0,81	0,75
	2	2	4	0,88	1,16	1,02
	2	1,9	3,8	1,01	0,81	0,82
	1,9	1,9	3,61	0,83	1,23	1,02

	1,9	1,9	3,61	1,33	1,52	2,02
	1,9	2	3,8	1,38	1,3	1,79
Negative control: (Aquadest)	1,9	1,9	3,61	1,39	1,51	2,10
	1,7	1,8	3,06	0,68	1,26	0,86
	1,6	1,9	3,04	1,33	1,45	1,93

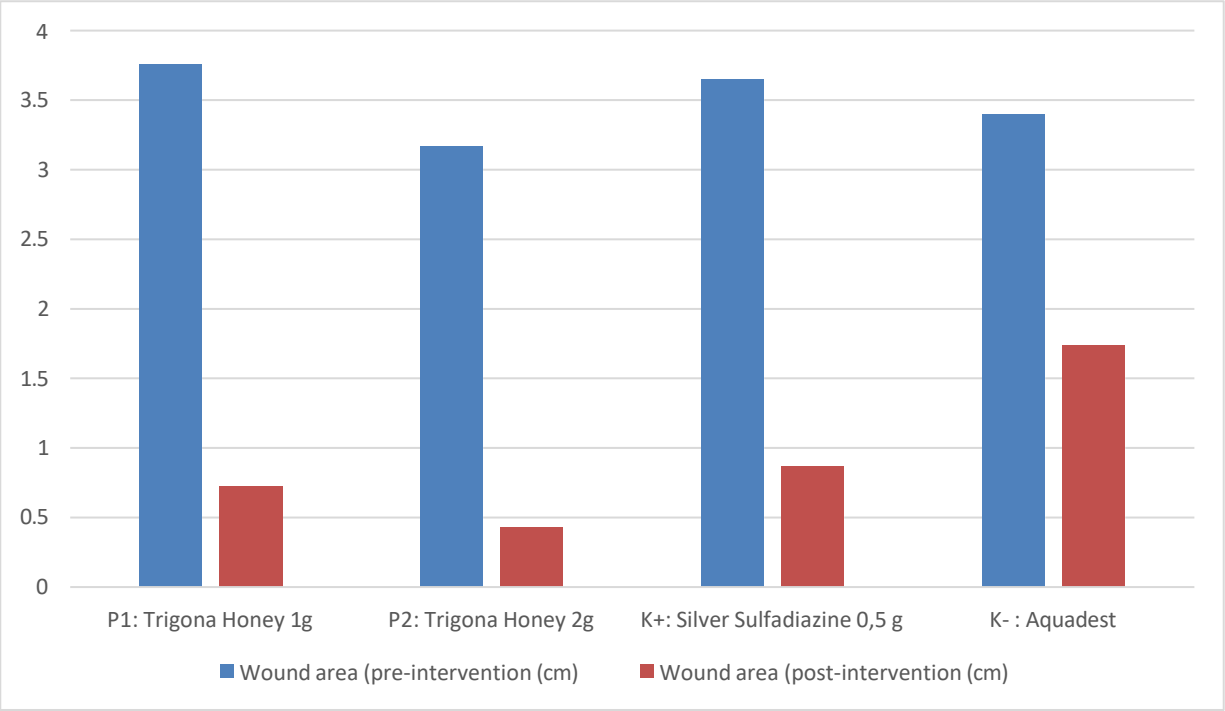

Diagram 1. Average value of burn area at 2 measurement times

The following are the changes in the average burn area in all groups at pre- and post-intervention. In the 2-gram per day honey group, the pre-intervention was 3.7 cm, while the post-intervention was 0.4 cm, resulting in burn healing of around 3.3 cm. In the 1-gram per day honey group, the pre-intervention was 3.1 cm, while the post-intervention was 0.7 cm, resulting in burn healing of around 2.4 cm. In the positive control group, the pre-intervention was 3.6 cm, while the post-intervention was 0.8 cm, resulting in burn healing of around 2.8 cm. In the negative control group, the pre-intervention was 3.4 cm, while the post-intervention was 1.7 cm, resulting in a burn healing of around 1.7 cm. If ranked, the average healing area from the best is the 2-gram honey group, followed by the positive control group (silver sulfadiazine), then the 1-gram honey group, and finally the negative control group (aquadest).

Table 2. Descriptive; Wound Area (Post-Intervention)

Groups	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Trigona Honey 1 g	5	0,7200	0,19481	0,08712	0,4781	0,9619	0,47	0,96
Trigona Honey 2 g	5	0,4280	0,34325	0,15351	0,0018	0,8542	0,22	1,03
Positive control (silver sulfadiazine 0,5 g)	5	0,8680	0,14272	0,06383	0,6908	1,0452	0,73	1,02
Negative control (aquadest)	5	1,7400	0,50522	0,22594	1,1127	2,3673	0,86	2,10
Total	20	0,9390	0,58509	0,13083	0,6652	1,2128	0,22	2,10

Table 3. Test of Within-Subjects Effects

Measure: Wound Area

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	65,556	1	65,556	369,165	0,000
	Greenhouse-Geisser	65,556	1,000	65,556	369,165	0,000
	Huyn-Feldt	65,556	1,000	65,556	369,165	0,000
	Lower Bound	65,556	1,000	65,556	369,165	0,000

Based on the repeated-ANOVA statistical test, the P value obtained for Greenhouse-Geisser = 0.000, it can be concluded that there is a significant difference at 2 different measurement times (before and after intervention).

Table 4. Wound Area (Post-Intervention)

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	4,779	3	1,593	14,770	0,000
Within Groups	1,726	16	0,108		
Total	6,504	19			

Followed by a one-way ANOVA test on measuring the area of burns after intervention to see the differences between the treatment group and the control group, in Between Groups the P value was 0.000 (<0.05) so it could be concluded that Trigona Spp Honey. effective as a burn therapy.

Table 5. Post Hoc Tests (Multiple Comparisons)
Dependent Variable: **Wound Area (Post-Intervention)**

Turkey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Trigona Honey 1 g	Trigona Honey 2 g	0,29200	0,20770	0,514	-0,3022	0,8862
	Positive control	-0,14800	0,20770	0,891	-0,7422	0,4462
	Negative control	-1,02000*	0,20770	0,001	-1,6142	-0,4258
Trigona Honey 2 g	Trigona Honey 1 g	-0,29200	0,20770	0,514	-0,8862	0,3022
	Positive control	-0,44000	0,20770	0,189	-1,0342	0,1542
	Negative control	-1,31200*	0,20770	0,000	-1,9062	-0,7178
Positive control	Trigona Honey 1 g	0,14800	0,20770	0,891	-0,4462	0,7422
	Trigona Honey 2 g	0,44000	0,20770	0,189	-0,1542	1,0342
	Negative control	-0,87200*	0,20770	0,003	-1,4662	-0,2778
Negative control	Trigona Honey 1 g	1,02000*	0,20770	0,001	0,4258	1,6142
	Trigona Honey 2 g	1,31200*	0,20770	0,000	0,7178	1,9062
	Positive control	0,87200*	0,20770	0,003	0,2778	1,4662

The difference in group averages can be seen in the Multiple Comparisons table (by looking at the * sign), the improvement in the area of burns in the Treatment group 1 is significantly different from negative controls group, the improvement in the area of burns in the Treatment group 2 is significantly different from negative controls group, the improvement in the area of burns Positive controls group is significantly different from negative controls group and negative controls group is significantly different from all groups. Statistically, the mean difference between groups 1 and 2 was not significantly different (p=0.514). This means that administering 1 g and 2 g had the same effect.

Figure 1. The process of shaving, cleaning and administering topical anesthesia (lidocaine prilocaine 5%)

Figure 2. Preparation for the induction of burns using a metal plate

Figure 3. Measuring the area of burns before administering honey and silver sulfadiazine intervention

Figure 4. The process of treating burns using honey and also silver sulfadiazine ointment

Figure 5. Measurement of burns after administering honey intervention and silver sulfadiazine ointment

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LAPORAN HASIL UJI
Report of Analysis
No : 24018924 / LHU / BBLK-MKS / VII / 2024

Nama Customer / *Customer Name*	:	dr. NUR FADHILLAH KHALID M. BIOMED	
Alamat / *Address*	:	BTN Minasa Upa D 11/4	
Jenis Sampel / *Type of Sample (S)*	:	Madu Trigona	
No. Sampel / *No. Sample*	:	24018924	
Tanggal Penerimaan / *Received Date*	:	18 Juli 2024 / *July 18, 2024*	
Tanggal Pengujian / *Test Date*	:	18 Juli 2024 s/d 24 Juli 2024 / *July 18, 2024* to *July 24, 2024*	

HASIL PEMERIKSAAN

No / No	Parameter / *Parameters*	Satuan / *Units*	Hasil Pemeriksaan / *Test Result*	Spesifikasi Metode / *Method Specification*
1	Flavonoid	mg/kg	149,35	Spektrofotometrik
2	Polifenol	mg/kg	405,73	Spektrofotometrik
3	Alkaloid	mg/kg	123,54	Spektrofotometrik
4	Vitamin A	mg/L	< 0,04	Spektrofotometrik
5	Vitamin C	mg/kg	400,89	Spektrofotometrik
6	Calsium (Ca)	mg/L	61,86	AAS
7	Kalium (K)	mg/L	33,45	AAS

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DP/084/BBLKM - Mks; Rev.3; 15 Februari 2024

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LAPORAN HASIL UJI
Report of Analysis
No : 24018924 / LHU / BBLK-MKS / VII / 2024

Nama Customer / *Customer Name*	:	dr. NUR FADHILLAH KHALID M. BIOMED	
Alamat / *Address*	:	BTN Minasa Upa D 11/4	
Jenis Sampel / *Type of Sample (S)*	:	Madu Trigona	
No. Sampel / *No. Sample*	:	24018924	
Tanggal Penerimaan / *Received Date*	:	18 Juli 2024 / *July 18, 2024*	
Tanggal Pengujian / *Test Date*	:	18 Juli 2024 s/d 24 Juli 2024 / *July 18, 2024* to *July 24, 2024*	

HASIL PEMERIKSAAN

No / No	Parameter / *Parameters*	Satuan / *Units*	Hasil Pemeriksaan / *Test Result*	Spesifikasi Metode / *Method Specification*
1	pH	-	4,22	IKM/KKT/149/BBLKM-MKS
2	Besi (Fe)	mg/L	2,6184	SM APHA 23rd Ed.,3111 B, 2017
3	Mangan (Mn)	mg/L	4,6085	SM APHA 23rd Ed.,3111 B, 2017
4	Seng (Zn)	mg/L	0,7412	SM APHA 23rd Ed.,3111 B, 2017

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DP/084/BBLKM - Mks; Rev.3; 15 Februari 2024

Figure 6. Examination nutritional contents of Trigona honey Spp.

Discussion

Trigona honey has various active substances that are beneficial for health. The following is the

composition and characteristics of honey which play a role in wound healing, including, flavonoids have antioxidant, anti-inflammatory and antimicrobial activity. Phenolic acid have antioxidant and antimicrobial activity. Amino acids have anti-inflammatory activity. Vitamins A, C and E have antioxidant activity. The sugar content (fructose, glucose and sucrose) is related to osmolality, the osmotic effect causes drainage of lymph fluid. Besides that, pH (degree of acidity) is < 4, the acidic nature of honey causes more oxygen supply from the circulation. And enzymes content (glucose oxidase and catalase), these enzymes are naturally produced by bees in making honey which is useful as an antibacterial. [6,7,9,12-15]

In Hendy & Lister's (2019) research entitled Level of Effectiveness in Healing Grade IIA Burns by Giving Honey and Nebacetin Ointment to White Rats (Rattus Norvegicus), the results showed that honey was effective in healing burns. This is shown by the results of wound healing in the group given honey once a day which healed in 7 days, given honey three times a day healed in 10 days, the group given topical nebacetin gel once healed in 9 days and in the control group healed in 13 days. This study is in line with the results of our study that honey is effective in healing wounds.[18]

The antioxidant and anti-inflammatory effects work by accelerating the rate of healing and modulating the immune response, increasing granulation tissue and epithelialization, reducing the amount of exudate and sterilizing wounds, stimulating leukocytes to release cytokines and growth factors needed for tissue repair and disrupting the process of inflammatory amplification by ROS (reactive oxidative stress). Then for the antimicrobial effect, honey has broad spectrum performance (honey is able to inhibit the growth of gram-positive or negative germs, as well as aerobic or anaerobic germs), effective against germs that are resistant to antibiotics such as Pseudomonas, MRSA (methicillin-resistant Staphylococcus aureus), coagulase negative Staphylococci, VRE (vancomycin-resistant Enterococci), Acinetobacter baumannii, and Stenotrophomonas maltophilia. Apart from that, honey also has other advantages compared to antibiotics that there is no decrease in bacterial sensitivity to honey after long-term use and it is effective against bacteria hidden in biofilms so honey is known to have anti-resistance. Due to the high sugar content in honey (Fructose, Glucose and Sucrose) it causes an increase in osmolarity which will attract bacterial intracellular fluid, so that ultimately plasmolysis occurs. The content of hydrogen peroxide, a chemical compound that is formed slowly by the enzyme glucose oxidase, which is naturally added by bees during honey production, is also useful as an antibacterial.[19-21]

The positive control active substance that we use is silver sulfadiazine, which is a sulfonamide and has broad antimicrobial activity. This drug acts on the cell walls and membranes of gram-positive and gram-negative bacteria. Apart from that, sulfadiazine is also effective as an antifungal. Silver sulfadiazine acts bactericidally by increasing cell wall permeability through interference with DNA replication, direct modification of cell membrane lipids, and/or the formation of free radicals. The mechanism of action of this drug is to release silver ions, slowly and in a controlled manner, when interacting with body fluids containing sodium chloride in the wound area. Then, the ionized silver atoms will accelerate the formation of disulfide

bonds, change protein structure, and inactivate thiol-containing enzymes. Silver ions can also change DNA, there by interfering with bacterial replication and transcription. Silver is a biocide that can bind to a fairly broad target. Silver ions can bind to nucleophilic amino acids, causing denaturation and enzyme inhibition. With this mechanism, silver ions will bind to the membrane surface and proteins, and cause proton leakage in the membrane. This will result in cellular death.[22-27]

Sulfadiazine is a competitive inhibitor of paraaminobenzoic acid (PABA) bacteria. Inhibited PABA will inhibit bacterial dihydropteroate synthesis, the ability of bacteria to form folic acid which is useful for DNA synthesis, so the bacteria will die. Silver sulfadiazine is useful for treating infections caused by burns, decubitus ulcers and wounds that occur due to injuries to the fingertips or abrasions. The results of this experiment are that silver sulfadiazine is also effective in healing burns due to the mechanism of action that has been explained. [22-27]

Conclusion

The findings of this study confirm that Trigona spp. honey. effective as a burn wound therapy in white Sprague Dawley rats. Flavonoid, phenolic acid, amino acids, vitamins A,C,E, enzymes (glucose oxidase and catalase), characteristic of pH<4, sugar content (fructose, glucose and sucrose), and minerals contribute to faster wound closure with mechanism of action as antioxidant, anti-inflammatory, and antimicrobial. Future studies should explore the long-term effects of Trigona spp. honey, evaluate the efficacy dan adds another parameter to see the wound healing.

Conflicts of Interest

There is no conflict of interest.

Funding sources

This research was funded by the UMI Waqf Foundation and had no influence on the research process or results.

Acknowledgments

The authors would like to thank the Chancellor of UMI who has facilitated this research and other parties who have contributed.

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