Original Article

Comparative Efficacy of Manuka Honey and its Combination with Fusidic Acid in Surgically Inflicted Methicillin Resistance *Staphylococcus aureus* Infected Wounds in Rabbits

Muhammad Masood Tahir[1], Zarreen Sajjad[2], Muhammad Saqib[3] and Fatima Sajjad[3]

[1]Pioneer Pets Hospital, Rawalpindi, Pakistan.

[2]National Veterinary Laboratory, Pakistan.

[3]Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan.

drmmt1992@gmail.com

Abstract

Wound healing is a complex mechanism that involves the recruitment and pragmatic proliferation of cells at the site of injury and it may be delayed due to many reasons including wound infection. Topical use of antimicrobials/antibiotics is one of the rationales for wound care. However, the development of resistance to antimicrobials is an emerging and serious problem for clinicians all over the globe. The present study was, therefore, planned to evaluate the comparative efficacy of Manuka honey (MH) alone and in combination with fusidic acid (FA) in the treatment of surgically inflicted full thickness MRSA infected wounds in rabbits. Adult male rabbits (n=30) were randomly divided into 3 groups (A to C) of equal size. A set of full-thickness skin wounds (3 on each rabbit) were inflicted on the dorsolateral aspects of the lumber region of each rabbit. Then infection was induced with 10 μl of 0.5 McFarland turbidity standard suspension of MRSA and waited for 24 hours to let the infection develop. Wounds in groups A and B were respectively treated with MH and FA, whereas, group C received a combined therapy comprising a mixture of MH and FA in equal proportions. The wound healing was evaluated in terms of the rate of wound contraction, healing time, and histopathological evaluations. The data was then subjected to statistical analyses using analysis of variance (ANOVA) considering it significant at $p<0.05$ and highly significant at $p<0.01$.

Keywords: Fusidic Acid, Manuka Honey, MRSA (Methicillin Resistant *Staphylococcus aureus*), McFarland

Citation. Tahir, MM, Sajjad, Z, Saqib, M, Sajjad, F. Comparative Efficacy of Manuka Honey and its Combination with fusidic Acid in Surgically Inflicted Methicillin Resistance *Staphylococcus aureus* Infected Wounds in Rabbits. J Vet Med Res., 2024; 31(2): 27–34. https://doi.org/10.21608/jvmr.2024.308922.1105 .

Article History: **Received**: 19-Jan-2025 **Accepted**: 20-Feb-2025

1. Introduction

Wound refers to any discontinuity of skin or superficial layer of any organ. Cutaneous wounds are most commonly encountered in animals and humans throughout the world. Depending on the type and intensity of trauma and surgical procedure, two types of wounds viz., open and closed are seen.

Open wounds are more serious and life threatening owing to the risk of bacterial infection and fluid loss (Bowler, 2003; Gardner et al., 2001). In response to the wound, the competent host (body) mediates a process of healing that encompasses various complex physiological phases of hemostasis, inflammation, and proliferation (development of granulation tissue and remodeling). Among normal healthy individuals, all these events of healing occur at an optimum rate (Kwon et al., 2007). Within a few hours after the injury, granulation tissue is formed at the site of injury, and the lining of the residual tissue starts the re-epithelialization and subsequently covers the granulation tissue over the site of injury (Stadelmann et al., 1998).

The emergence of Multi-drug resistant (MDR) strains has reduced the antimicrobial options. Combined antimicrobial therapy is recommended because two antimicrobials with different mechanisms of action prevent the development of resistance (Rahal, 2006). This strategy also reduces cost and side effects and provides augmented efficacy due to synergistic. Similarly, natural products (honey for one) have shown potential for use with antimicrobials to increase the antimicrobial effects and to reduce the emergence of resistance (Wagner and Ulrich-Merzenich, 2009). Unattended open wounds invariably harbor bacteria, which can be lethal when the pathogens are MDR.

Skin infections are mostly caused by Staphylococci (resident flora of skin) and Enterobacter (*E. coli*, an environmental pathogen). However, nosocomial bacterial pathogens (Methicillin-resistant *Staphylococcus au-*

reus 'MRSA' and *Pseudomonas aeroginosa*) are highly dangerous as they survive under extreme antimicrobial pressure and are invariably resistant to several antimicrobials. Both pathogens cause serious wound infections, especially in immunocompromised patients (Jenkins and Cooper, 2012a).

Manuka honey is a type of mono-floral honey that is obtained from *Leptospermum scoparium* in Australia and New Zealand and is an effective treatment for the healing of wounds in animals. Honey used for this purpose must not contain any sort of residue or particles from pesticides or herbicides (Creemers and Bosma, 2006). Honey is a material of high nutritive value; comprising 20 types of different sugars, eight different kinds of vitamins, 11 different minerals, 16 kinds of amino acids, and also contains many enzymes (Fattahi Bafghi et al., 2007). Among the medicinal properties, honey carries angiogenic, antibacterial, and anti-inflammatory activities and is reported to enhance the generation of granulation tissue, speed up the process of epithelialization, prevent and reduce the formation of scars, and help in alleviating pain.

Such properties are because of the generation of hydrogen peroxide, the very high osmolarity of honey, and the presence of certain phytochemical substances in the nectar of plants (Khan et al., 2007). Honey has both medicinal as well as nutritive value. Apart from being highly nutritious, it enhances the process of wound healing and produces a soothing effect particularly on burn wounds by reducing microbial activity and enhancing the process of re-epithelialization (Khiati et al., 2014). The antibacterial activity of Manuka honey is parallel to the scale value of 18 on the scale of phenol acid strength and makes this honey the strongest currently available medical honey as far as antibacterial activity is concerned (Sare, 2008).

2. Materials and methods

The present study was conducted on surgically inflicted full thickness skin wounds on the torso (lumber region) in adult male rabbits. Experiments were conducted under the guidance of the rules of the Institutional Review Board of the Ethical Committee of Animal Care at the University of Agriculture, Faisalabad, Pakistan.

2.1. Experimental Animals

Thirty locally bred, clinically healthy male adult rabbits, weighing between 1.5–2.5 kg were randomly divided into 3 groups, labeled A, B, and C having 10 animals each. Following randomization, all animals were housed and acclimatized for 2 weeks in the Laboratory Animal Facility of the Department. Animals were endowed with light and dark cycles in well-ventilated temperature maintained (~ 22-28°C) rooms. During the course of acclimatization, each rabbit was dewormed with ivermectin (400 microgram/kg) and received a 3-day prophylactic course of Amoxicillin (15 mg/kg subcutaneously) for pasturellosis. All these prophylactic tactics were completed a week before the commencement of the experimental trial (Awais, 2013).

2.1.1. Preparation of the Surgical Site

The skin and hair follicles are the prime medium to harbor bacteria, which in a real sense cannot be sterilized completely. Bacterial flora on the skin surface is responsible for the causation of wound-associated infections in animals. The chances of acquiring such infections during the infliction of surgical wounds can be reduced transiently to a comparatively safer level by the removal of hair, mechanical scrubbing, and using some germicidal solution. Considering all these things in mind, hairs from the surgical site and liberal surrounding area were clipped to reduce the chances of infection as much as possible without harming skin which might later on interfere with wound healing.

Close careful clipping of hair within the operative field is an absolute necessity in surgery (Awais, 2013). Therefore, an electric clipper was preferred over the ordinary razor for this purpose as abrasions caused by the conventional razor disrupt the topmost layers of skin and dent the body's first line of defense against bacterial invasion. A general guideline was supervised in each case while clipping the hairs properly on the torso (trunk; thoracolumbar) region. The clipped area was then given a general cleaning scrub, with soap and water followed by thorough rinsing. After completing the washing process, the entire clipped area was dried with an autoclaved clean towel. The operation field was then fashioned for aseptic surgery by spraying methylated spirit twice and tincture of iodine once after the other.

2.1.2. Pre-medication and Anesthesia

Each rabbit from all groups was pre-medicated by administering atropine sulphate@ 0.035 mg/kg body weight subcutaneously about half an hour before the surgical procedure. The animals were anesthetized by parenteral anesthesia via intramuscular route using a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg) (Lipman et al., 1990).

2.1.3. Positioning of Rabbit and Draping of Surgical Site

The rabbits were secured in sternal recumbency. Before surgical intervention, the area was cleaned and sanitized completely with iodine followed by alcohol swabbing. An effective draping system is considered mandatory and is an important barrier to contaminants from the surrounding skin and environment. Wet cloth drapes allow penetration of bacteria when dry and serve as a potent barrier. Keeping this fact in mind, a single square drape having a central square cut was used separately for each rabbit in this pursuit of research.

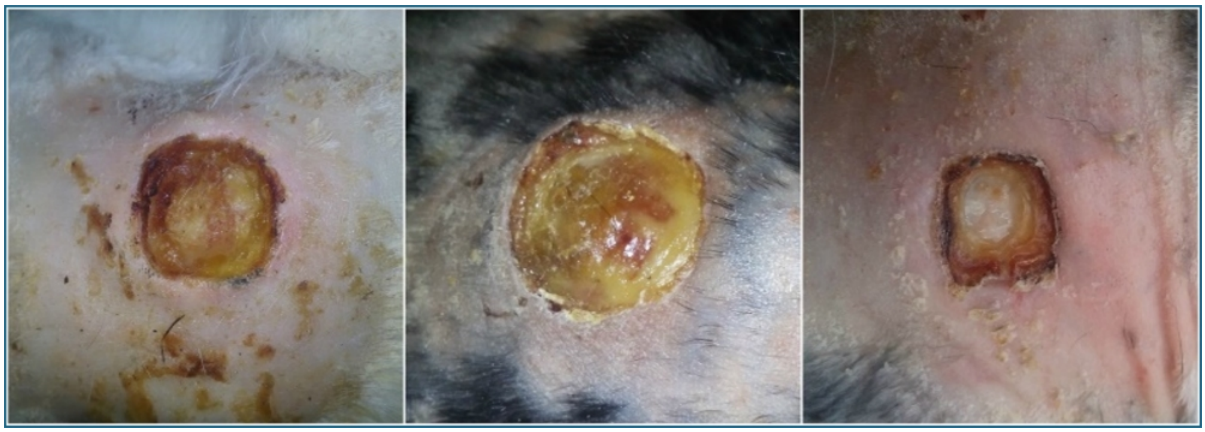

Figure 1: Wounds on the fifth day of treatment (Group A, B, and C from Left to Right).

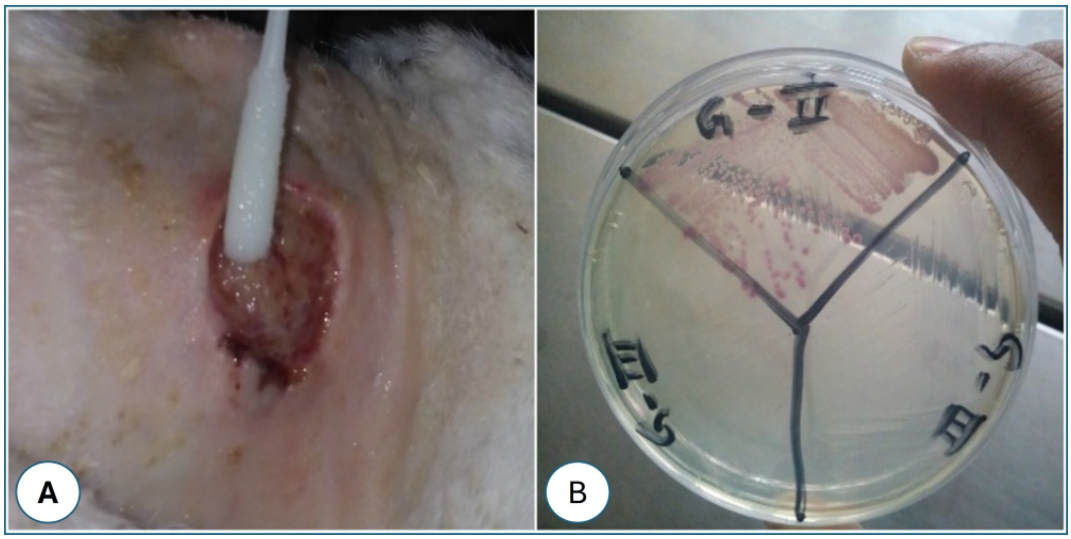

Figure 2: Swab collection from the wound (Left) Petri plate showing growth of MRSA on Nutrient Agar in group B (Right).

2.1.4. Surgical Infliction of Wounds and Identification Marks

Wounding area was marked with a permanent black marker using Vernier calipers. Three, 1×1 cm (1 cm^2) full-thickness skin wounds were created on the trunk comprising each rabbit in a triangular symmetry with an anterior wound on the dorsal midline and two rear wounds, one on either flank. The inter-wound distance was kept at 3 cm on either side. Full thickness wound was created on the trunk with the help of a #15 blade (Carbon Steel, Feather Safety Razor Co, Ltd. Japan), and the piece of skin from the wound site was removed by undermining the area with sharp-sharp scissors that exposed subcutaneous area. The subcutaneous fat and fascia were also removed and trunk.

A total of three wounds of similar dimensions were inflicted on the torso of each rabbit's dorsum. The wounds of group A designated as follows for the proper identification and to avoid repetition of treatment:

- **A-wF=** wound created on the midline of the thoracolumbar area on the front of the body
- **A-wRR=** wound created lateral to the thoracolumbar area on the right rear of the body
- **A-wLR=** wound created lateral to the thoracolumbar area on the left rear of the body

A similar naming was assigned to the wound on the subject of group B. The wounds were designated as follows:

- **B-wF=** wound created on the midline of the thoracolumbar area in front of the body
- **B-wRR=** wound created lateral to the thoracolumbar area on the right rear of the body
- **B-wLR=** wound created lateral to the thoracolumbar area on the left rear of the body

Similarly, identifications for group C wounds were as follows:

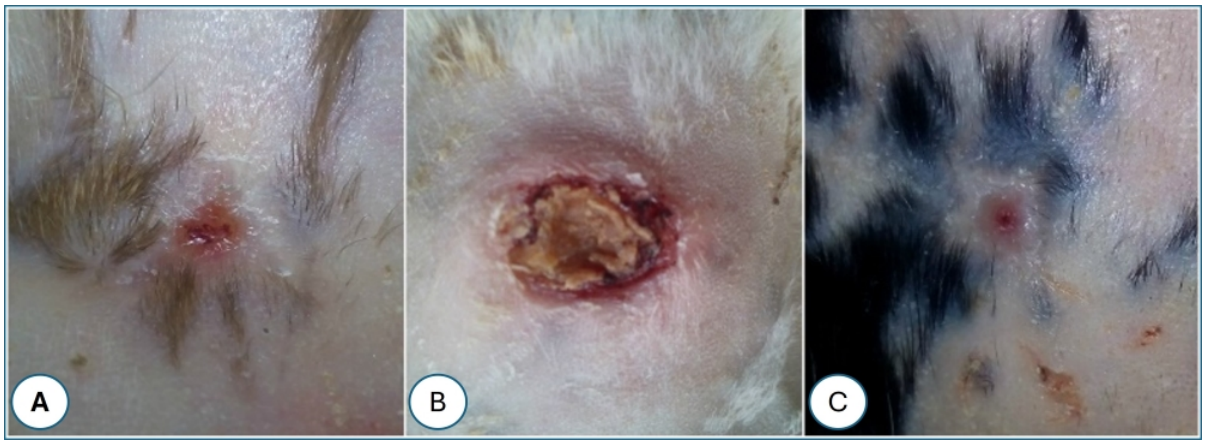

Figure 3: Wounds on the 18th day of treatment (Group A, B, and C from Left to Right).

Table 1: Wound Contraction Rate (cm) SE ± 0.01 (cm).

Days of Treatment	Group A	Group B	Group C
0	0.00	0.00	0.00
3	0.09	0.05	0.08
6	0.22	0.11	0.21
9	0.37	0.16	0.37
12	0.51	0.24	0.58
15	0.66	0.36	0.74
18	0.82	0.46	0.95
21	1.00	0.57	1.00
24	1.00	0.68	1.00
27	1.00	0.77	1.00
30	1.00	0.86	1.00
33	1.00	0.91	1.00
36	1.00	1.00	1.00

- **C-wF=** wound created on the midline of the thoracolumbar area on the front of the body
- **C-wRR=** wound created lateral to the thoracolumbar area on the right rear of the body
- **C-wLR=** wound created lateral to the thoracolumbar area on the left rear of the body

2.1.5. Induction and Development of Infection

After the surgical infliction of wounds, the infection was given with strain-35 of methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 33592) as described earlier (Roche et al., 2012). Each wound was given 10 μl of 0.5 McFarland Turbidity Standard suspension of MRSA in the center of each wound and then spread out using a pipette tip to cover the entire wound area (McFarland is a standard unit used for estimating the number of bacteria in suspensions, for calculating the opsonic index and vaccine production on the basis of transmittance and absorbance of light, where 0.5 McFarland is equivalent of 10^5 bacteria/ml of suspension). After the induction of infection, we waited 24 hrs for the development of infection before starting any treatment.

2.2. Experimental Protocol

The following treatment protocol was adopted for each of the groups:

- **Group A =** wounds were treated with Manuka honey (MH)
- **Group B =** wounds were treated with fusidic acid (FA)
- **Group C =** wounds were treated with a mixture of MH and FA

Wounds in group A were treated with topical application of Manuka honey alone right from the day next to the induction of infection. Similarly, wounds in group B were treated with topical fusidic acid (Fusidin® 2% fusidic acid by LEO Pharma Pvt. Ltd.) alone which served as a control group. The subjects in group C received a combination of MH and FA mixed in equal proportion, also applied topically on the wounds in the relevant group.

After the application of treatments, the wounds were left open. The treatments were applied once a day until the day of complete healing. In order to prevent licking by the other animals, all the rabbits were kept in separate cages. During the whole course of treatment, all

Table 2: Analysis of variance for wound contraction rate between group A vs group B vs group C.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	2	0.005	0.0025	33.784*
Error	27	0.002	0.000074	
Total	29	0.007		

*Highly significant (p-$value$<0.01).

Table 3: Analysis of variance for Wound contraction rate between group A vs group B.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	1	0.002	0.002	36.41*
Error	18	0.001	0.000055	
Total	19	0.003		

*Highly significant (p-$value$<0.01).

the rabbits were checked daily for general health conditions, any cross infection into the wounds, and other unspecified abnormalities.

2.3. Evaluation Parameters

2.3.1. Wound Contraction

The rate was estimated by tracing the edges of wounds on a piece of trace paper. These sketches of wound sizes were measured on graph paper (mm^2) and wound contraction values were presented in percentile (Awais, 2013).

2.3.2. Healing Time

Healing time refers to time (days) elapse between the creation of a wound and the complete regeneration and epithelization. It was estimated by the sum of daily observations until the scar fell off (Awais, 2013). The experiment was conducted from February 20, 2016 to April 19, 2016.

2.3.3. Statistical Analysis

The data thus obtained was subjected to analysis of variance using the SPSS program to discern the substantial effects of treatment considering the level of significance at P<0.05.

3. Results

The present study was planned and conducted to evaluate the efficacy of manuka honey alone and in combination with fusidic acid in the treatment of surgically inflicted full-thickness MRSA-infected wounds in adult rabbits. One experiment was carried out on 90 full thickness skin wound models in 30 rabbits and randomly divided into 3 equal groups with 30 in each. The study lasted over 60 days. All general physiological parameters (including body temperature, respiration rate, and heart rate) were checked frequently and were found within normal ranges. None of the animals showed any signs of anorexia, however, a remarkable increase in the water intake was observed in almost all rabbits which may also be correlated with the change in external weather, though the temperature of the animal house of maintained within a comfortable zone.

3.1. Wound Contraction Rate

The inward movement of the wound edges towards the center during the course of healing is inferred as contraction. Following the initial expansion during 2 days post-surgery, all wounds started to contract inward and this rate was measured in mm by tracing the edges of wounds on a tracing paper and then measuring it on a graph paper until the wound edges had completely closed (Figure 1 & Figure 3).

In order to counter-test the presence of active infection/infectious agent, we took random swabs from different wounds in different groups and cultured them onto a culture medium. After 24 hrs, we observed the presence of MRSA in group 2 swabs) and these results were in full accordance with the end results of healing time as well as showing the slowest healing in that very group (Figure 2). The results of wound contraction rates in different groups and their statistical comparison through Analysis of Variance (ANOVA) have been presented in Tables, Table 1-Table 5.

3.2. Healing Time

The time (days) elapse between the creation of the wound and the complete regeneration and re-epithelialization of wound edges is termed as healing time. The shortest mean wound healing time (18.75±0.425 days) was observed in the wounds included in group C (treated with a combination of manuka honey and fusidic acid) and in group A (Manuka honey alone) mean healing time was 21.1±0.459) and slowest healing (34.1±1.197 days) was recorded in group B (fusidic acid alone) (Table 4). Statistically, healing was significantly faster (P< 0.05) in group C than in groups A and B. The difference in healing time between groups A and C was comparatively less significant. The ANOVA on the healing time is presented in

Table 4: Analysis of variance for wound contraction rate between group A vs group C.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	1	0.001	0.001	18.94*
Error	18	0.001	0.000055	
Total	19	0.002		

*Significant (p-$value$<0.05).

Table 5: Analysis of variance for wound contraction rate between group B vs group C.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	1	0.005	0.002	97.18*
Error	18	0.001	0.000055	
Total	19	0.006		

*Highly significant (p-$value$<0.01).

Table 6.

3.3. Histopathological Evaluation

Biopsy specimens of full skin thickness were collected from the healed wounds on days 7 and 14 of the treatment and were preserved in 10% buffer formalin (v/v). After fixation, the biopsy specimens were subjected to blocking, cutting, sectioning by microtome, mounting on a slide, fixing, and slide staining processes. Then finally analyses were made for the deposition of collagen fibers, their arrangement, dermal and epidermal layers' thickness, fibroblast proliferation, re-epithelialization, and formation of new vascular beds in the healed tissues (Yadav et al., 2012). The wounds treated with a combination of MH and FA showed the highest collagen contents and maximum angiogenesis (Figure 4A) which were markedly better than the other two groups but the development of epidermis was observed best in the results of wounds treated with MH alone Figure 4B) than group B and C. Whereas, thin epidermis with some collagen fiber of blood vessels were observed in histopathological findings of wound tissue being treated with fusidic Acid alone Figure 4C).

4. Discussion

Perhaps for as long as the occurrence of a wound has been reported, both humans and animals the latest ways to aid and stimulate the healing process have been searching for. Modern research necessitates the searching for ways that stimulate the wound healing process and much of the information has been yielded concerning the common wound infections and infectious agents, their methods of control, the nature of the wound, and better methods of wound care and management than previously existing.

The wound healing process usually demands sustenance at three different levels: initially at enhancing the general support and resistance, then at accelerating the repair and regenerative phenomenon, and last but not the least, the therapeutic and nutritional processes (Gutteridge, 1993; Simon et al., 2006; Nisbet et al., 2010). Congregation of these needs was well facilitated by *Nigella sativa* honey. Histopathological examination revealed the commencement of the proliferative phase on the third day of the treatment group which was delineated by the formation of granulation tissue, depicting that *Nigella sativa* stimulated the synthesis of human interleukins and caused the alertness of macrophages.

Macrophages are capable of phagocytosing bacteria and serve as a second line of host defense (Haq et al., 1995). A number of chemotactic and many growth factors (Pakyari et al., 2013) were also released such as transforming growth factor (TGF), interleukin-1 (IL-1), fibroblasts growth factor (FGF) and epidermal growth factor (EGF) which were responsible to circumscribe the proliferative phase. The formation of collagen fibers was triggered by certain growth factors and growth hormones. This was also helpful in kindling the proliferation of fibroblasts leading to the development of granulation tissue, hastening through the re-epithelialization and collegenization. However, it was reported to delay granulation tissue formation and scar development. Besides that, the *Nigella sativa* oil contains some fatty acids that help in the construction of collagen contents sustaining the resilience of skin.

The significantly improved contraction rate in wounds treated with a combination of manuka honey and antibiotics was in accordance with the results of several in vitro studies on the synergistic activity of manuka honey with different antibiotics. Manuka honey enhanced the susceptibility of MRSA to oxacillin by down-regulating the *mecR1* gene known to enhance methicillin resistance in MRSA (Jenkins and Cooper, 2012b). Methylglyoxal (MGO) present in manuka honey showed an additive effect with Rifampicin and completely inhibited the growth of MRSA (Mandal and Mandal, 2011; Müller et al., 2013). A combination of Imipenum with manuka honey and Tetracycline with manuka honey showed synergistic effects in clearing MRSA growths in vitro (Jenkins and Cooper, 2012a).

Table 6: Analysis of variance for wound healing time.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Treatment	2	1367.150	683.575	1123.6849*
Error	27	16.425	0.608	
Total	29	1383.575		

*Highly significant (p-$value$<0.01)

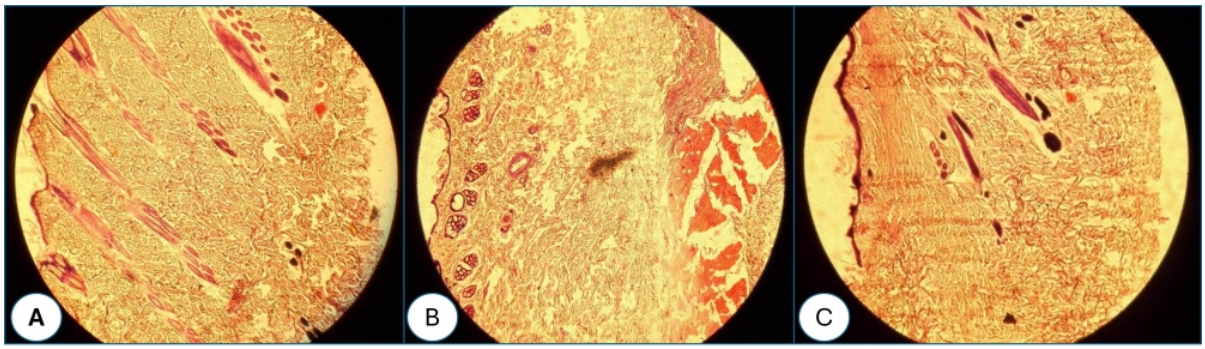

Figure 4: A photomicrograph showing (**A**) thin epidermis formation and sufficient presence of collagen fiber and some blood vessels in the dermis of healing wound tissue being treated with Manuka honey in group A. (**B**) the thin epidermis with some collagen fiber of blood vessels in healing wound tissue being treated with fusidic Acid in group B. (**C**) moderate to good thickness epidermis and high presence of collagen fiber in the dermis and blood vessels in the healing wound tissue being treated with a combination of Manuka Honey and fusidic Acid in group C.

Manuka honey when used alone, and in combination with fusidic acid, in both circumstances possesses anti-inflammatory properties. Therefore, in this way, they reduce the chances of inflammatory edema, improve sloughing off of devitalized tissue, attract macrophages for clearing the wound, act as an energy source for the local wound tissue, and mask the wound acting as a protective barrier against possible contaminations. The hyperosmolar and acidic nature of manuka honey confers to its antibacterial properties (Gupta et al., 1992; Sudhakar et al., 2003).

Histopathological evaluation in the current study revealed that group C of wounds (treated with a combination of manuka honey and fusidic acid mixed in equal proportions) showed the best development of epidermis among the three groups and also the greatest thickness of the skin layer from animals of other groups A and B (treated with manuka honey alone and fusidic acid alone respectively). However, the presence of collagen fibers was less significantly different in group C from that of group A, but still highly significant compared to group B. These results were in accordance with the findings of (Gupta et al., 1992; Sudhakar et al., 2003).

4.1. Conclusion

From the current study, it was concluded that manuka honey in combination with fusidic acid not only improves wound healing by promoting the wound contraction rate but also reduces the antibiotic resistance of MRSA. Therefore, it is recommended to use manuka honey in a mixture of equal proportions with fusidic acid for the treatment of fresh-cut infected wounds in animals and humans is very efficient and can be used with utmost confidence without any fear of side effects.

Article Information

Ethical Approval. Experiments were conducted under the guidance of the rules of the Institutional Review Board of the Ethical Committee of Animal Care at the University of Agriculture, Faisalabad, Pakistan.

Funding. The research received no external funding.

Conflict of Interest. The authors declare no conflict of interest.

References

Awais, M., 2013. Effects of a mixture of Acacia honey andpovidone on the healing of full-thickness skin wounds in normal anddexamethasone treated rabbits. Master thesis. Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad.

Bowler, P., 2003. The 105 bacterial growthguideline: re assessing its clinical relevance in wound healing. Ostomy Wound Manage 49, 44–53.

Creemers, T., Bosma, W.J., 2006. Honey based wound ointment for wound healing and skin disorders with animals. Journal of Veterinary and Animal Sciences 28, 112–117.

Fattahi Bafghi, A., Yavari, M., Hossein Zadeh, J., 2007. Usefullness of honey from the islamic point of view and evaluation of its effects on cutaneous leishmaniasis wounds in Balb/c rats invitro. Journal of Shahid Sadoughi University of Medical Sciences 4, 32–37. URL: https://jssu.ssu.ac.ir/article-1-1295-en.html.

Gardner, S.E., Frantz, R.A., Doebbeling, B.N., 2001. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair and Regeneration 9, 178–186. 10.1046/j.1524-475x.2001.00178.x.

Gupta, S., Singh, H., Varshney, A., Prakash, P., 1992. Therapeutic efficacy of honey in infected wounds in buffaloes. AgricultIndian Journal of Animal Research 62, 521–523.

Gutteridge, J.M., 1993. Free radicals in disease processes: a compilation of cause and consequence. Free radical research communications 19, 141–158. URL: https://www.ncbi.nlm.nih.gov/pubmed/8244084.

Haq, A., Abdullatif, M., Lobo, P.I., Khabar, K.S., Sheth, K.V., al Sedairy, S.T., 1995. *Nigella sativa*: effect on human lymphocytes and poly-

morphonuclear leukocyte phagocytic activity. Immunopharmacology 30, 147–155. 10.1016/0162-3109(95)00016-m.

Jenkins, R., Cooper, R., 2012a. Improving antibiotic activity against wound pathogens with manuka honey in vitro. Plos One 7, e45600. 10.1371/journal.pone.0045600.

Jenkins, R.E., Cooper, R., 2012b. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. The Journal of Antimicrobial Chemotherapy 67, 1405–1407. 10.1093/jac/dks071.

Khan, F.R., Ul Abadin, Z., Rauf, N., 2007. Honey: nutritional and medicinal value. International Journal of Clinical Practice 61, 1705–1707. 10.1111/j.1742-1241.2007.01417.x.

Khiati, B., Bacha, S., Aissat, S., Ahmed, M., 2014. The use of algerian honey on cutaneous wound healing: a case report and review of the literature. Asian Pacific Journal of Tropical Disease 4, S867–S869. 10.1016/S2222-1808(14)60748-9.

Kwon, A.H., Qiu, Z., Hirao, Y., 2007. Topical application of plasma fibronectin in full-thickness skin wound healing in rats. Experimental Biology and Medicine 232, 935–941. URL: https://www.ncbi.nlm.nih.gov/pubmed/17609510.

Lipman, N.S., Marini, R.P., Erdman, S.E., 1990. A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. Laboratory animal science 40, 395–398. URL: https://www.ncbi.nlm.nih.gov/pubmed/2166867.

Mandal, M.D., Mandal, S., 2011. Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine 1, 154–160. 10.1016/S2221-1691(11)60016-6.

Müller, P., Alber, D.G., Turnbull, L., Schlothauer, R.C., Carter, D.A., Whitchurch, C.B., Harry, E.J., 2013. Synergism between medihoney and rifampicin against methicillin-resistant *Staphylococcus aureus* (MRSA). Plos One 8, e57679. 10.1371/journal.pone.0057679.

Nisbet, H.O., Nisbet, C., Yarim, M., Guler, A., Ozak, A., 2010. Effects of three types of honey on cutaneous wound healing. Wounds: A compendium of clinical research and practice 22, 275–283. URL: https://www.ncbi.nlm.nih.gov/pubmed/25901517.

Pakyari, M., Farrokhi, A., Maharlooei, M.K., Ghahary, A., 2013. Critical role of transforming growth factor beta in different phases of wound healing. Advances in wound care 2, 215–224. 10.1089/wound.2012.0406.

Rahal, J.J., 2006. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. Clinical Infectious Diseases 43 Suppl 2, S95–9. 10.1086/504486.

Roche, E.D., Renick, P.J., Tetens, S.P., Carson, D.L., 2012. A model for evaluating topical antimicrobial efficacy against methicillin-resistant *Staphylococcus aureus* biofilms in superficial murine wounds. Antimicrobial Agents and Chemotherapy 56, 4508–4510. 10.1128/AAC.00467-12.

Sare, J.L., 2008. Leg ulcer management with topical medical honey. British journal of community nursing 13, S22, S24, S26 passim. 10.12968/bjcn.2008.13.sup4.30930.

Simon, A., Sofka, K., Wiszniewsky, G., Blaser, G., Bode, U., Fleischhack, G., 2006. Wound care with antibacterial honey (Medihoney) in pediatric hematology-oncology. Supportive Care in Cancer 14, 91–97. 10.1007/s00520-005-0874-8.

Stadelmann, W.K., Digenis, A.G., Tobin, G.R., 1998. Physiology and healing dynamics of chronic cutaneous wounds. American Journal of Surgery 176, 26–38. 10.1016/s0002-9610(98)00183-4.

Sudhakar, A., Sugimoto, H., Yang, C., Lively, J., Zeisberg, M., Kalluri, R., 2003. Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by alpha v beta 3 and alpha 5 beta 1 integrins. Proceedings of the National Academy of Sciences of the United States of America 100, 4766–4771. 10.1073/pnas.0730882100.

Wagner, H., Ulrich-Merzenich, G., 2009. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 16, 97–110. 10.1016/j.phymed.2008.12.018.

Yadav, K.H., Kumar, J.R., Basha, S.I., Deshmukh, G., Ravi, G., Santhamma, B., 2012. Wound healing activity of topical application of *Aloe vera* gel in experimental animal models. International Journal of Pharma and Biosciences 3, 63–72. URL: https://www.ijpbs.net/details.php?article=1277.