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EFFECT OF APIS HONEY ON ANASTOMOTIC WOUND HEALING IN RATTUS MODELS

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ABSTRACT

A wound is a disturbance in the normal structure and function of the epidermis. The epidermis is considered as the first line of defense and protection against trauma. Wound healing is a complex process with many interdependent immunological and pathophysiological mediators to restore the cellular integrity of the damaged tissue (Molan *et al.,* 2015). With the emergence of drug-resistant bacteria, many antimicrobial agents have become ineffective in wound treatment, and many failures in current wound treatment methods have been reported. For this reason, alternative therapies have been sought, one of which is the use of honey as a wound treatment agent (Hixon *et al.* 2019). The use of honey has recently gained clinical popularity for possible use in wound treatment and in regenerative medicine (Hixon *et al.,* 2018). A study was conducted to assess antibacterial and the wound-healing activity of Apis honey using excision wound models in rats. Most of the honey samples with various dilutions have proved to possess, significant antibacterial potency against selected bacterial isolates such as *Bacillus cereus (ATCC 31443), Bacillus subtilis (ATCC 32441), Burkholderia glumae (ATCC 25813), Erwinia nigrifluens (ATCC 21922), Escherichia coli (ATCC 25891), Klebsiella sp. (ATCC 31482), Pseudomonas aeruginosa (ATCC 287858) and Staphylococcus aureus (ATCC 6538).* Also different formulations of honey were used and rats were treated topically. The area of epithelization was found to increase, followed by an increase in wound contraction, skin-breaking strength, and tissue granulation. Our experiments con□rm that honey can aid wound healing when applied topically in several rat models of wound healing.

INTRODUCTION

Natural honey is composed of around 82% of water, carbohydrates, proteins, phytochemicals, antioxidants, and minerals. It has been proven that few of the ingredients that determine the biological and medical potential of this substance are likely to vary among the various types of honey. The sugars in honey include, fructose (38.2%), glucose (31.2%), disaccharides and some other tri-saccharides and higher saccharides (9%) and sucrose (0.7–1%)" (Visavadia *et al.,*2008). Honey containing a wide range of active compounds, including flavonoids, organic acids, phenolic acid, vitamins, and enzymes, may improve wound healing. The deposition of fibroblasts and collagen formation may also be promoted by the amount of amino acids found in honey (Dryden *et al.,* 2014).

Natural honey is a viscous fluid; its jelly consistency creates a surface layer over the wound that inhibits the entrance of bacteria and protects the wound from dehydration (Molan 2001). Its high sugar content creates a higher osmotic gradient that pulls fluid up through the subdermal tissue and offers an additional glucose source for flourishing cellular components in the wounded area (Sundoro *et al.,* 2012). The low pH of honey increases tissue oxygenation, while free radicals, which lead to tissue damage, are removed by flavonoids and aromatic acids (Speer *et al.,* 2015).

According to the international guidelines on the proper use of antimicrobials in medicine, honey and other alternative therapeutics were used for the treatment of skin lesions on animal models (Olofsson *et al.,* 2016). Honey exerts bacteriostatic and bactericidal actions (Vandamme *et al.,* 2013; Al-Nahari *et al.,* 2015; Girma *et al.,* 2019).

MATERIALS AND METHODS

Study areas

The present study areas of Karnataka were of different biogeographical regions of Coorg (12.3375° N, 75. 8069° E) district.

Procurement of *Apis* honey samples

One hundred and thirty six *Apis* honey samples of *Apis florea, Apis mellifera, Apis cerana Apis dorsata,* were harvested from various geographical areas of Coorg, Karnataka during 2019-2020. Each honey sample was first filtered with a sterile mesh to remove debris. All the samples were collected and transported in sterile sealed bottles or screwed cups with authentic labels. Four replications of bottles for each sample were kept under storage at 2-8 ^{0}C until tested as per the method proposed by Nzeako and Hamdi (2000).

Determination of antibacterial potency of honey samples

Collection of bacterial isolates

The test clinical control isolates used in the present study were collected during 2019. The clinical isolates were identified based on the standard microbiological technique (Chess brough, 1998). The bacterial strains, *Bacillus cereus (ATCC 31443), Pseudomonas aeruginosa (ATCC 287858), Bacillus subtilis (ATCC 32441), Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 25891), Burkholderia glumae (ATCC 25813), Erwinia nigrifluens (ATCC 21922), Klebsiella sp. (ATCC 31482)* were used to determine the antibacterial activity of each sample of honey.

Culturing of bacterial strains

The collected clinical control microorganisms maintained in the laboratory on Nutrient Agar(Hi-Media) by slant–streak technique for further pure cultures (Mackie, 1999). The slants with strains were stored in deep freezer at 4 ^{0}C to -70 ^{0}C.

Under aseptic conditions, pure colonies of bacterial isolates from slants were picked with an inoculating loop and suspended in 3-4 ml of nutrient broth in sterile test tubes and incubated for 24 h at 36-37°C (Andargarchew et al., 2004).

The microbial organisms were simultaneously tested for sensitivity against the selected antibiotics (Kanamycin) on nutrient agar Hi-medium plates. Organisms showing inhibition zones equal to, or greater than that of the control organisms were regarded as sensitive to honey samples(Patton *et al.,* 2006).

Further, the antibacterial activity of honey and experiments on wounds of the experimental animal models (*Albino* rats) with five replicates of each was subjected to F-test and analysis of variance to determine the significant levels at 5% (p<0.05).

Pharmacological evidence of antibacterial potency on wounds of *Albino* rats

The present study was designed to evaluate the pharmacological effects of various types of honey on infected burn and incision wounds of Albino rats.

Twenty five male *Albino* rats weighing 250-350 g each were used in the present study. The rats were kept in the animal unit at one week prior to initiation of the study. The rats were given commercial pellet and water throughout the study to ensure stabilization of their good health. Rats were anesthetized with an injection of Ketamine (50 mg/kg) and Xylazine (5mg/kg). Under anesthesia, the back of both sides of the body were shaved . Following this procedure, rats were returned to their cages for 24 h to allow any edema caused by the shaving procedure to recede.

The wound site was prepared following the excision wound model (Glowania *et al.,* 1987; Adikwu and Alozie, 2007). Initially, the rats were anesthetized as described above and a circle of diameter of 15mm was marked one each right side of the thigh of animals skin surface, and the skin was gently dissected out. The area was measured immediately by tracing out the wound area using a sterile transparent tracing paper and the area was recorded.

Treatment was initiated only after 2 days of excision as the wound was exposed for the bacterial infection. After 2 days of incision, the wound was swabbed with potent concentration of honey with high range of antibacterial property. Simultaneously, the wound area of each animal was measured while the animals were under anesthesia on the days of post surgery. Each application was evaluated in 5 rats per group and results shown were a mean of 5 determinations (Adikwu and Alozie, 2007& Leong *et al.*, 2012). A group *Albino* rats with incision but without treatment were used as control.

Measurement of wound contraction

The excision wound margin was traced after wound creation by using transparent paper and the respective area was measured using a graph paper. Wound contraction was measured at every 2 days' interval, until complete wound healing, and expressed in percentage of the healed wound area (Speer *et al.,* 2015 The evaluated surface area was then used to calculate the percentage of wound contraction, taking the initial size of wound 500mm2 as 100%, by using the following formula:

$$\%\text{Wound contraction} = \frac{\text{Initial wound size} - \text{Specific } (n^{th}) \text{ day wound size}}{\text{Initial wound size}} \times 100$$

The data obtained from wound healing of incision wounds was subjected to analysis of Mean Standard Deviation.

RESULTS

The present study found significant intrinsic antibacterial activity of *Apis* honey samples collected from the study regions of Coorg, Karnataka. The clinical evaluation of honey showed significant visible differences in the process of wound healing on the animal models of rats.

Antibacterial efficacy of honey

Most of the honey samples with various dilutions have proved to possess, significant antibacterial potency against selected bacterial isolates such as *Bacillus cereus (ATCC 31443), Bacillus subtilis (ATCC 32441), Burkholderia glumae (ATCC 25813), Erwinia nigrifluens (ATCC 21922), Escherichia coli (ATCC 25891), Klebsiella sp. (ATCC 31482), Pseudomonas aeruginosa (ATCC 287858) and Staphylococcus aureus (ATCC 6538).*

The antibacterial sensitivity with inhibitory zones that were formed in the cultures against the selected bacterial strains showed significant variations with the Coorg honey samples. The Coorg honey of *Apis florea* showed highest antibacterial activity at 75 to 95 per cent dilutions (Fig.1) against *Pseudomonas aeruginosa (ATCC 287858)* with 10.8 ± 0.97 mm and the lowest being 6.0 ± 0.18 mm. However, the least sensitivity range was recorded for *Erwinia nigrifluens (ATCC 21922)* with 6.0 ± 0.32 mm.

The Coorg honey of *Apis mellifera* showed highest antibacterial activity at 75 to 95 per cent honey dilutions (Fig.1) against *Pseudomonas aeruginosa (ATCC 287858)* with 7.8 ± 0.18 mm and the lowest being 6.5 ± 0.08 mm at 75 to 95 per cent dilutions (Fig.1) against *Bacillus cereus* (ATCC 31443), 6.2 ± 0.95 mm against *Bacillus subtilis* (ATCC 32441). The other bacterial species showed complete resistance at all dilutions of honey.

The Coorg honey of *Apis cerana* showed highest antibacterial activity at 75 to 95 per cent honey dilutions against *Pseudomonas aeruginosa (ATCC 287858)* with 9.3 ± 0.05 mm and the lowest being 6.1 ± 0.34 mm against *Staphylococcus aureus (ATCC 6538) (Fig.1).* The other bacterial species showed resistance to all the dilutions of honey samples.

The Coorg honey of *Apis dorsata* showed highest antibacterial activity at 75 to 95 per cent honey dilutions (Fig.1) only against *Pseudomonas aeruginosa* (ATCC 287858) with 6.0 ± 0.18 mm. The other bacterial species showed resistance to all the dilutions of honey samples.

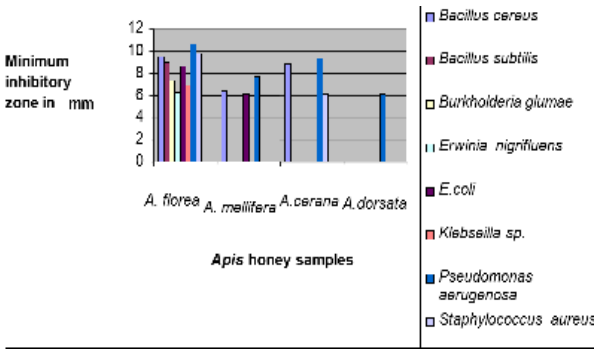

Fig.1. Showing minimum inhibitory zones at 75 to 95 % honey (dilutions) samples from Coorg during 2019-2020.

Antibacterial potency and wound healing property of honey on experimental animal models

The honey samples of the present study areas that retained antibacterial potency at varied dilutions against control isolates, were used in the wound healing of experimental *Albino* rats. The wound healing experiments on the *Albino* rats showed significant variations.

Table.1. Showing mean number of days (Mean ± Standard Deviation) for the healing of wounds on experimental animal models in vitro.

Type of Wound	Mean No. of Days					
	Control	Treatment With Kanamycin	Treatment with Apis honey Coorg			
			A. florea	*A. cerana*	*A. mellifera*	*A. dorsata*
Excision	20 ± 1.07	10±1.45	12 ±1.06	13 ±1.02	14 ±1.91	15 ±1.31
	Percentage of wound Healing on 10^{th} day					
	49± 1.30	100± 1.42	88± 1.33	81± 1.61	79± 1.38	74± 1.59

Significant at p< 0.005 levels

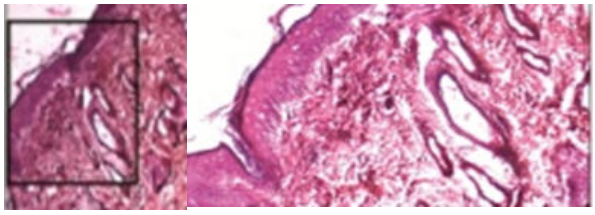

Fig.2. Wound tissue specimen after complete healing of excision wound, stained with hematoxylin and eosin.

DISCUSSIONS

Antibacterial potency is the effect influenced by the agent preferably the chemicals that inhibit or slow down the growth of bacteria in the given media. A laboratory demonstration of antibacterial activity was first carry out by Dold *et al.* (1937). He also gave the concept of "inhibine" to the substance which inhibits the growth bacteria. Honey has been demonstrated in many studies to have antibacterial effects, attributed to its high osmolarity (Sugar content), low pH, high hydrogen peroxide, high moisture content, high ash content and other uncharacterized compounds. Low pH alone is inhibitory to many pathogenic bacteria (Molan, 1997 & Alvarez *et al.,* 2014). Hydrogen peroxide was identified as the major source of antibacterial activity in honey by White *et al.* (1988). Thus, all the factors such as low pH, high sugar content and peroxide content are combattingly responsible for antibacterial activity of medicinally important and potent honey (Olofsson *et al.,* 2016). A study of 345 samples of New Zealand honey found antibacterial activity of diluted honeys (Molan, 1997). He had also suggested the influence of phytochemical origin and geographical origin of honey in the antibacterial activity. In the present study, most honey samples of Coorg exhibited potent antibacterial activity.

The Coorg honey of *Apis florea* showed highest antibacterial activity at 75 to 95 per cent dilutions against *Pseudomonas aeruginosa (ATCC 287858*) with 10.8 ± 0.97 mm and the lowest being 6.0 ± 0.18 mm. The least sensitivity range was recorded for *Erwinia nigrifluens (ATCC 21922)* with 6.0 ± 0.32 mm. The Coorg honey of *Apis mellifera* showed highest antibacterial activity at 75 to 95 per cent honey dilutions against *Pseudomonas aeruginosa (ATCC 287858*) with 7.8 ± 0.18 mm and the lowest being 6.5 ± 0.08 mm at 75 to 95 per cent dilutions against *Bacillus cereus (ATCC 31443),* 6.2 ± 0.95 mm against *Bacillus subtilis (ATCC 32441)*. The Coorg honey of *Apis cerana* showed highest antibacterial activity at 75 to 95 per cent honey dilutions against *Pseudomonas aeruginosa (ATCC 287858)* with 9.3 ± 0.05 mm and the lowest being 6.1 ± 0.34 mm against *Staphylococcus aureus (ATCC 6538*). The Coorg honey of *Apis dorsata* showed highest antibacterial activity at 75 to 95 per cent honey dilutions only against *Pseudomonas aeruginosa (ATCC 287858)* with 6.0 ± 0.18 mm. Earlier studies by Albaridi, (2019), Anand *et al.,* (2019), Matzen *et al.* (2018) and Adeleke *et al.* (2006) mentioned the use of diluted honey in controlling the bacterial growth and the dilutions could be confirmed through *in vivo* and clinical studies. The present findings are in accordance with previous studies reported that different honey types possess different efficacies and mechanisms against the same bacteria (Cebrero *et al.,* 2020; Al-Masaudi, 2020, Al-Masaudi *et al.,* 2017 & 2020; Lu *et al.,* 2014; Carnwath *et al.,* 2014). Nzeako and Hamdi (2000) reported antibacterial activity of *Pseudomonas, Acinobacter* and *Staphylococcus* was noticed at 40 per cent dilutions of Saudi Arabian honey. Andargarchew *et al.* (2004) reported antibacterial activity against *E.coli, S.aureus, P. aeruginosa, S.shiga, S.typhi, P. vulgaris, K.aerogenes* and *P.mirabilis* at various dilutions of *A.melliferan* honey. French *et al.* (2005) reported antibacterial activity of honey against coagulase negative *Staphylococci*. Noori *et al.* (2005) reported against *Streptococcus, E.coli* and *Staphylococuss aureus*. Mitra *et al.* (2000) reported antibacterial activity of honey against *S.aureus, E.coli, P.aeruginosa,* and *Klebsiella*.

The usage of honey in the clinical fields is presumed to be one of the ancient methods but renewed the development in the recent days (Molan, 2006). The excision wounds are injury made to the integument that causes cellular death, capillary damage in varying degrees and coagulation of proteins. The loss of protective function of the skin as a barrier to micro organisms results in infection. The bacteria contaminate the wound surface and begin multiply and proliferate in the wound area (the excellent culture medium) (Molan, 2009). Honey with high osmolarity, low pH and high peroxide content favors the outflow of fluid from wound tissue, aiding cleansing, reducing edema and decreasing pain. The pure honey and diluted honey, when applied to wounds, permits movement of water from an area of high concentration, to an area of low concentration, thus contributing to the cleaning of wounds. (Georgina, 2005). And also, the movement of fluid from underlying tissue and capillaries in response to this osmotic pull will lead to improvements in the increased levels of dissolved oxygen and nutrients. Thus, the nutrient content of honey stimulates the cell growth and provide energy for the dividing cells on the surface of wounds (Molan PC. 2006). Honey along with wound healing prevents scar formation (Subrahmanyam, 1992).

In this study, the excision wounds were assessed by gross inspection of epithelialisation and wound healing. The high potency of *Apis florea* honey from Coorg district showed 12 ± 1.06 days for healing of incision wounds upon the treatment than the control animals (20 ± 1.07 days). Similar findings were reported by Georgina (2005), Molan (2006), Bilsel *et al.* (2002) and Adikwu and Alozie (2007). Bangroo *et al.* (2005) reported wound healing in human patients using honey. Owen *et al.* (2001) and Vandamme *et al.,*(2013) reported systemic wound healing using honey on human patients. Agata *et al.* (2004) studied the burn wound healing in pigs. Mitra *et al.* (2000) reported the ophthacare brands using honey in various groups of Rabbit population. Molan (1997) studied wound healing in mice, rats and buffalo calves.

CONCLUSION

Variations in the antibacterial activity could be attributed by the honey bee species, floral varieties even it is collected from the same geographical region hence identification of appropriate honey type to control the specific bacterial growth is required. Further deciphering of phytochemicals in the effective honey variety is important in order to use the honey against specific pathogens.

REFERENCES

1. Adeleke, O.E., Olaitan, J.O. & Okpekpe, E.L. 2006. Comparative antibacterial activity of honey and gentamicin against Escherichia coli and Pseudomonas aeruginosa. Ann Burns Fire Disasters. 19, 201-204.
2. Adikwu, M.U. And Alozie, B.U. 2007. Application of snail mucin dispersed in detarium gum gel in wound healing . Sci. Res. Essays. 2 (6): 195-198.
3. Agata Kabala-Dzik, Rafal Stojko, Ewa Szaflarsha-Stojko, Irena Wroblewska-Adamek, Artur Stojko, Jerzy Stojko And Barbara Stawiarska-Pieta. 2004. Influence of honey Balm on the rate of scare formation during experimental burn wound healing in Pigs. Bull. Vet. Inst. Pulawy. 48: 311-316.
4. Albaridi, N.A. 2019. Antibacterial potency of honey. International Journal of Microbiology, 2464507.
5. Al-Masaudi, S.B. 2020. The antibacterial activities of honey. Saudi Journal of Biological Sciences. 28, 2188- 2196.
6. Al-Masaudi, S.B., Al-Nahari, A.A.M., Abd El-Ghany, E.S.M., Barbour, E., Al Muhayawi, S.M., Al-Jaouni, S., Azhar, E., Qari, M., Qari, Y.A. & Harakeh, S. 2017. Antimicrobial effect of different types of honey on Staphylococcus aureus. Saudi Journal of Biological Sciences, 24; 1255–1261.
7. Al-Nahari AA, Almasaudi SB, Abd El-Ghany el SM, Barbour E, Al Jaouni SK, Harakeh S. 2015. Antimicrobial activities of Saudi honey against Pseudomonas aeruginosa. Saudi J Biol Sci.;22 (5):521–5.
8. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, Mazzoni L, Giampieri F. 2014. The composition and biological activity of honey: a focus on Manuka honey. Foods.;3 (3):420–32.
9. Anand, S., Deighton, M., Livanos, G., Morrison, P.D., Pang, E.C.K. & Mantri, N. 2019. Antimicrobial activity of Agastache honey and characterization of its bioactive compounds in comparison with important commercial honeys. Frontiers in Microbiology, 10.
10. Andargarchew Mulu, Belay Tassema. And Fetene Derbie. 2004. In vitro assessment of antimicrobial potential of honey on common human pathogens, Ethiop. J. Health Dev. 18 (2): 107-111.
11. Bangroo, A. K., Ramji Khatri And Smith Chauhan. 2005. Honey dressing in Pediatric burns, J. Indian Assoc. Pediatr. Surg. 10 (3): 172-175.
12. Bilsel, Y., Bugra, D., Yammer, S., Bulut, T., Cevikbas, U. And Turkoglu, U. 2002.

Could Honey have a place in Colitis Therapy? Effects of Honey, Prednisolone and Disulfiram on Inflammation, Nitric oxide and Free - Radical formation. Dig. Surg. 19:306-312.
13. Cebrero, G., Sanhueza, O., Pezoa, M., Baez, M.E., Martínez, J., Baez, M. & Fuentes, E. 2020. Relationship among the minor constituents, antibacterial activity and geographical origin of honey: A multifactor perspective. Food Chemistry, 126296.
14. Chess Brough, M. 1998. Medical Laboratory Manual for Tropical countries . Vol. II, Microbiol. 196 -205.
15. Dold, H., Du, D.H. And Dziao, S.T. 1937. Nachweis antibakterieller hitze- und lictemp finlicher Hemmungsstoffe Inhibine im Naturhonig bling (Detection of antibacterial heat and light sensitive substance in natural honey) Z Hyg. Infektionskr, 120:155-167.
16. Dryden M, Goddard C, Madadi A, Heard M, Saeed K, Cooke J. 2014. Using antimicrobial Surgi honey to prevent caesarean wound infection. Br J Midwifery.;22 (2):111–5.
17. French ,V. M., Cooper, R. A. And Molan, P. C. 2005. The antibacterial activity of honey against coagulase - negative Staphylococci, J. Antimicrobial Chemotherapy. 56:228-231.
18. Georgina Gethin. 2005. Can honey contribute to healing chronic wounds? Practice Nurse. J. Irish Practice Nurses Assoc. 7 (3):9-11.
19. Girma A, Seo W, She RC. Antibacterial activity of varying UMF-graded Manuka honeys. PLoS One. 2019;14 (10):e0224495.
20. Glowania, H. J., Raulin, C. And Swoboda, M. 1987. Effect of Chamomile on wound healing-a clinical double-blind study ,Z. Hautkr 62:162-171.
21. Hixon KR, Klein RC, Eberlin CT, Linder HR, Ona WJ, Gonzalez H. 2019. A critical review and perspective of honey in tissue engineering and clinical wound healing. Adv Wound Care.;8 (8):403–15.
22. Hixon KR, Lu T, Carletta MN, McBride-Gagyi SH, Janowiak BE, Sell SA. 2018. A preliminary in vitro evaluation of the bioactive potential of cryogel scaffolds incorporated with Manuka honey for the treatment of chronic bone infections. J Biomed Mater Res B Appl Biomater.;106 (5):1918–33.
23. Leong AG, Herst PM, Harper JL. 2012. Indigenous New Zealand honeys exhibit multiple anti-inflammatory activities. Innate Immun.;18(3):459–66.
24. Lu, J., Turnbull, L., Burke, C.M., Liu, M. Carter, D.A., Schlothauer, R.C., Whitchurch, C.B. & harry, E.J. 2014. Manuka-type honeys can eradicate biofilms produced by Staphylococcus aureus strains with different biofilm forming abilities. Peer J, 2, e326.
25. Mackie Mc Cartney. 1999. Practical Medical Microbiology. In:R.S. Miller, S.G.B Amyes, 4th Ed. Lab. Control of Antimicrobial Therapy. pp 151-178.
26. Matzen, R.D., Leth-Espensen, J.Z., Jansson, T., Nielsen, D.S., Lund, M.N. & Matzen, S. 2018. The antibacterial effect in vitro of honey derived from various Danish flora. Dermatology Research and Practice, 2018.
27. Mitra, S. K., Sundaram, R., Venkataranganna, M. V., Gopumadhavan, S., Prakash,N, S., Jayaram, H. D. And Sarma, D. N. K. 2000. Anti-inflammatory, Antioxidant and Antimicrobial activity of ophthacare Brand¸ an Herbal eye drops. Phyto Med. 7 (2):123-127.
28. Molan P, Rhodes T. 2015. Honey: a biologic wound dressing. Wounds.;27 (6):141–51.
29. Molan PC, 2009. White R. Honey in modern wound management. Aberdeen, UK:Wounds UK Ltd.
30. Molan, P. C. 1997. The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity, Bee Wld. 73: 59-76.
31. Molan, P. C. 2006. The Evidence supporting the use of Honey as Wound Dressing. Lower Extremity Wounds 5 (1): 40-54.
32. Noori, S. Al – Waili, Mohammed Akmal, Faiz S. Al – Wailli, Khelod M. Saloom, And Amjed. 2005. The Antimicrobial potential of honey from United Arab Emirates on some microbial isolates. Med. Sci. Monitt. 11 (12):433-438.
33. Nzeako, B. C. And Hamdi, J. 2000. Antimicrobial potential of honey on some microbial isolates, Med. Sci. 2: 75-79.
34. Olofsson TC, Butler É, Lindholm C, Nilson B, Michanek P, Vásquez A. 2016. Fighting off wound pathogens in horses with honeybee lactic acid bacteria. Curr Microbiol.;73 (4):463–73.
35. Owen A. Moore, Lesley. A. Smith, Fiona Campbell, Kate Seers, Henry J. Mcquay And R. Andrew Moore. 2001. Systematic review of the use of honey as a wound dressing. Abstract Notes, Pain Research Unit, Uni. Oxford. UK. pp 22-34.
36. Patton, T., Barrett, J., Brennan, J.& Moran, N. 2006. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J Microbiol Methods. 64;84–95.
37. Speer SL, Schreyack GE. 2015. Manuka honey: a tissue engineering essential ingredient. J Tissue Sci Eng.;6(2):1.
38. Subrahmanyam, M. 1992. Storage of skin grafts in honey. Lancet 34: 63-64.
39. Sundoro A, Nadia K, Nur A, Sudjatmiko G, Tedjo 2012. A Comparison of physical–chemical characteristic and antibacterial effect between Manuka honey and local honey. J Plastik Rekonstruksi.;1:3.
40. Vandamme L, Heyneman A, Hoeksema H, Verbelen J, Monstrey S. 2013. Honey in modern wound care: a systematic review. Burns.;39 (8):1514–25.
41. Visavadia BG, Honeysett J, Danford M. 2008. Manuka honey dressing: an effective treatment for chronic wound infections. Br J Oral Maxillofac Surg.;46 (8):696–7.
42. White, J. W., Subers, M. H. And Schepartz, A. I. 1988. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochem. Biophys. Acta, 73: 57-70.