Selection of Honey for Use as a Wound Dressing

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Summary

The use of honey as a dressing material for infected wounds is becoming of increasing interest as more reports of its effectiveness are published and as alternative treatments are sought for wounds infected with antibiotic-resistant strains of pathogens. But there appears to be a general lack of awareness of the very large variation that is found in the potency of the antibacterial activity of honey, which may explain the differences reported in the speed of clearance of infection from wounds dressed with honey.

Mostly, the antibacterial activity of honey is due to enzymically generated hydrogen peroxide, which is decreased by exposure of the honey to heat or light during processing or storage, or inactivated by substances in the nectar from some plants. In addition to this, there are non-peroxide antibacterial substances from the nectar of certain plants. These are usually only minor components, but in some honeys from some Leptospermum *species (manuka and jellybush) there is a high level of non-peroxide antibacterial activity. This may be significant clinically, as the catalase activity present in wounds has the potential to inactivate the hydrogen peroxide produced in honey.*

In the absence of data from a comparative clinical trial, the rational approach to selecting a honey for use in wound care would be to choose a honey with a high level of both hydrogen peroxide and non-peroxide antibacterial activity. Because there is such a marked variation in honeys, even within a floral type, laboratory testing is necessary to establish the antibacterial potency.

Introduction

Honey is known to have been applied to wounds over 4,000 years ago [1] and has continued to be used as a wound dressing throughout the intervening years [2, 3]. Although honey went out of common use as a wound dressing with the advent of antibiotics, in the past decade or two, it has been rediscovered by medical practitioners as a therapy for wounds [1]. This increase in interest in recent years is perhaps due to the problem of dealing with antibiotic-resistant strains of pathogens: honey has been found to be effective on wounds infected with multi-resistant bacteria [4].

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The clinical literature on the use of honey as a wound dressing has recently been reviewed [5]. The numerous papers all report very good results being obtained, with many reports of rapid clearance of infection from wounds.

Several authors are of the opinion that it is just the sugar content of honey that is responsible for its antibacterial effect [6-13], yet there have been many microbiological studies carried out that have shown that, in some honeys at least, there are other components present with a much more potent antibacterial effect [14]. This additional antibacterial activity is due to enzymically generated hydrogen peroxide and, in some honeys, to plant-derived substances as well [14].

Almost all of the clinical reports fail to recognise that there are marked differences in the antibacterial properties of honeys, although this has been recognised for over 40 years [15]. Honey is produced from many sources and its antimicrobial activity varies markedly with its floral origin and its processing [16] but clinical reports have rarely specified the type of honey that has been applied to infected wounds. An editorial commentary in *Archives of Internal Medicine* in 1976 on medical folklore [17] placed "honey from selected geographic areas" in the category of "worthless but harmless substances".

This is in contrast to the wisdom of physicians in past millennia, who specified particular types of honey be used to treat particular ailments. Dioscorides (c.50 AD) stated that a pale yellow honey from Attica was the best, being "good for all rotten and hollow ulcers" [18] and Aristotle (384-322 BC), discussing differences in honeys, refers to pale honey being "good as a salve for sore eyes and wounds" [19]. Present day folk medicine also recognises differences in honeys: the strawberry tree honey of Sardinia is valued for its therapeutic properties [20]; in India, lotus honey is said to be a panacea for eye diseases [21]; honey from the Jirdin valley of Yemen is highly valued in Dubai for its therapeutic properties [22]; manuka honey has a long-standing reputation in New Zealand folklore for its antiseptic properties (K. Simpson, personal communication).

The clinical importance of the antibacterial activity of honey

The high osmolarity of substances such as honey, saturated sugar syrups and sugar pastes is sufficient to inhibit microbial growth[23]. When used as dressings, dilution of these solutions by wound exudate reduces the osmolarity to a level that ceases to control infection, especially if wounds are infected by *Staphylococcus aureus* [24, 25], a common wound pathogen that is very osmotolerant. Such wounds are effectively rendered sterile by honey [26-29] because of its additional antimicrobial components.

Although both sugar and honey give a covering that can be easily removed from the surface of the wound without damaging regenerating tissue and both create the moist environment needed for optimal wound repair, sugar is found to be limited in its ability to suppress microbial growth. Bose [6] has commented that frequent changes of sugar dressings are necessary to maintain a therapeutic action, compared with only twice-daily changes of honey dressings. Some clinicians have found it necessary to supplement the inhibitory action of sugar by the addition of antiseptics to the dressing material [30-32]. However, this has the potential of delaying healing, as antiseptics cause tissue damage [33, 34]. Honey has the inhibitory action of its sugar content supplemented with its endogenous antiseptic components. Numerous reports have shown this to be harmless to wound tissues [5].

Measurements have been made of the dilution occurring from the uptake of water from surrounding tissues in an abdominal wound packed with sugar [24]. It was found that the water activity increased within 4 hours to a value of 0.897. This is equivalent to a concentration of honey of 22 per cent (v/v). Growth of *S. aureus* will occur at water activity above 0.86 [35]. Honeys from the middle of the normal range of antibacterial activity have been found to be capable of preventing growth of the major wound infecting species of bacteria when diluted 10 fold or more and with *S. aureus* could be diluted more than 50 fold [36].

The effect of the additional antibacterial activity in honey can be seen where honey and sugar have been used comparably. In an experimental study conducted on burns created on the skin of pigs [37], there were fewer bacterial colonies seen histologically in wounds treated with honey compared with wounds treated with sugar. There were also fewer micro pustules in the neo-epidermis and fewer bacteria seen in the eschar of the honey treated wounds compared with those treated with sugar. The only clinical report of comparative effectiveness of honey and sugar was one case of a discharging deep pressure sore not responding to various treatments, including dressing with sugar, which was completely healed in 6 weeks by dressing with honey [38].

It is the antibacterial activity additional to the osmolarity that varies in different honeys. But because there has been little information given on the honeys used in the various clinical studies reported, it cannot be known how much correlation there is between antibacterial potency and effectiveness in clearing infection from wounds. However, there have been some large differences in results reported between hospitals when there has been little difference in the treatment. In some of the clinical studies on the usage of honey as a dressing for infected wounds, a rapid clearance of infection has been reported across a range of different types of wound, all the wounds becoming sterile in 3-6 days [27, 29], 7 days [26, 39, 40] or 7-10 days [28]. In other studies the honey was not as effective. There have been reports of bacteria still present in the wound after 2 weeks [41, 42], 3 weeks [43-45], and 5 weeks [4]. Differences in the amount of the endogenous antiseptic components in the honeys used could be responsible for the differences in results.

The potency of the antibacterial activity of the particular honey used would be thought to be a major clinical consideration as infection is one of the most common impediments to wound healing [46]. In a recent review of the numerous reports on the successful usage of honey as a dressing on infected wounds, it was noted that many authors attributed the effectiveness of honey in part to its antibacterial properties [5]. The clinical significance of the antibacterial activity of honey can be seen in reports of honey being effective on wounds not responding to conventional therapy with antibiotics and antiseptics [4, 26, 38, 41, 42, 45, 47]. But little regard has been given to the level of antibacterial activity in the honeys used in any of the clinical reports, although it has been suggested by Postmes [48] in a report on testing *in vitro*, that for evaluation of the results of a clinical trial of honey the antibacterial activity of the batch of honey might be important. However, the clinical significance of the additional antibacterial activity in honey will be known with certainty only if a clinical trial is conducted to compare dressings of sugar and selected honeys [49].

Variation in antibacterial activity of honey

Not all honeys have the additional components that give honey an antibacterial activity greater than that due to the sugar content. The variation in potency of the antibacterial activity of honey was recognised more than 40 years ago in testing *in vitro*. A method was described for indicating the potency called the 'inhibine number', the number of steps along a five step dilution series maintaining an inhibitory concentration of each honey [15].

Much wider ranges of MIC values (the minimum concentration of honey necessary for complete inhibition of bacterial growth) have been reported in comparisons of a variety of samples of honey tested against single species of bacteria: from 25 to 0.25 per cent (v/v) [50]; >50 to 1.5 per cent (v/v) [51]; 20 to 0.6 per cent (v/v) [52]; 50 to 1.5 per cent (v/v) [53].

A survey of 345 samples of New Zealand honeys from 26 different floral sources found a large number with low activity (36 per cent of the samples had activity near or below the level of detection in an agar diffusion assay), the rest having almost a Gaussian distribution over a 30 fold range of activity [54]. A survey of 340 samples of Australian honeys from 78 different floral sources found 68.5 per cent of the samples had activity below the level of detection in an agar diffusion assay (C. Davis, Queensland Department of Primary Industries: personal communication).

Honey is produced from many floral sources and its antimicrobial activity varies markedly with its origin as well as with the way it has been processed and stored [16]. The antibacterial activity of honey is primarily due to hydrogen peroxide which is enzymically generated when honey is diluted› effectively giving a 'slow release delivery' of this antiseptic when honey is diluted by wound fluid [55].

The large differences in potency of antibacterial action found between different honeys are due mostly to differences in the amount of hydrogen peroxide produced. The enzyme that produces it is easily destroyed by exposure to heat and to light [56, 57]. Also there are components of some nectars which help inactivate the enzyme and others which break down hydrogen peroxide [55]. But some of the variation is due to the existence of additional non-peroxide antibacterial factors which various researchers have reported finding in some honeys [16], presumably derived from particular nectar sources. These are seen as antibacterial activity persisting in honeys treated with catalase to remove the hydrogen peroxide activity [54, 58-61].

It would be expected that the catalase in wound exudate would inactivate at least part of the hydrogen peroxide produced in the bed of a wound dressed with honey. The catalase which is present in plasma, at a mean level of 6.9 units/ml [62], (i.e. 6.9 µmol of hydrogen peroxide removed per minute per ml) is of high enough activity to potentially prevent any accumulation of hydrogen peroxide in a situation where honey is diluted by wound exudate. The maximum rate of production of hydrogen peroxide observed in a number of samples of honey has been reported to be 0.08 [59], 0.18 [63] and 0.31 [51] µmol of hydrogen peroxide per gram of honey per minute.

The catalase present in exuding plasma in a wound may also be augmented by catalase released from dead cells. In testing *in vitro*, it was found that 5 per cent blood added to the culture medium completely removed the antibacterial activity of the various honeys being used [48]. But the activity of catalase is low with physiological levels of hydrogen peroxide [64], so until studies are conducted *in vivo* it cannot be known for certain how effectively antibacterial is the hydrogen peroxide produced by honey on a wound.

Because of the possible inactivation of hydrogen peroxide from honey on a wound, there has been an interest in honeys with high levels of the non-peroxide antibacterial activity that is not removed by catalase. Although this type of antibacterial activity in honey is generally low in comparison with that due to hydrogen peroxide [14], one of the 26 floral types sampled in the survey of New Zealand honeys, manuka (*Leptospermum scoparium*), was found to have an exceptionally high level of non-peroxide antibacterial activity [54].

A similar finding has been made in the survey of Australian honeys in respect of honey from an unidentified *Leptospermum* species, jellybush (C. Davis, Queensland Department of Primary Industries: personal communication). The non-peroxide antibacterial activity of manuka honey has been tested against seven major wound infecting species of bacteria in comparison with that due to hydrogen peroxide [36]. Honeys with activity that was in the middle of the normal range for each type of activity were used.

Overall, there was no difference in effectiveness between the two types of antibacterial activity but individual species differed in their relative sensitivity. The most common wound pathogens, *S. aureus* and *Escherichia coli*, were more sensitive to the non-peroxide factor. The MIC values, as per cent v/v, were 1.8 and 3.7 for the manuka honey, and 4.9 and 7.1 for the other honey, respectively. With *Pseudomonas aeruginosa* the non-peroxide factor of manuka honey was less effective (MIC 10.8 per cent c/f 6.8 per cent), but this study was carried out with catalase added to the manuka honey so that just the non-peroxide factor was involved.

In a study of the same manuka honey against 20 isolates of *Pseudomonas* from infected wounds, in which catalase was not added, the mean MIC was found to be 6.9 per cent (v/v), ranging from 5.5 per cent to 8.7 per cent [65]. The MIC of this manuka honey without catalase added was found to be 1 per cent (v/v) for some multi-resistant strains of MRSA [66]. Although jellybush honey probably contains the same non-peroxide antibacterial component as that in manuka honey, it remains to be established chemically that this is so, or microbiologically that the sensitivity of bacteria to these two types of honey is the same.

Selection of honey

Like with all other honeys, the potency of the antibacterial activity of manuka honey varies a lot from sample to sample [67]. More than half of the very large number of samples of manuka honey and jellybush honey that have been tested have been found to have no detectable non-peroxide antibacterial activity (K. Allen, University of Waikato; C. Davis, Queensland Department of Primary Industries: personal communications).

It is therefore important if using these honeys for wound care that the honey is not randomly selected but is selected after laboratory testing of the potency of its non-peroxide antibacterial activity. Although any honey will have an antibacterial action because of the high osmolarity due to its sugar content, where the honey gets diluted by wound exudate a honey of low potency may not maintain an effective concentration of antibacterial activity. Also, a honey of higher potency would give a more effective diffusion of the antibacterial substance into infected body tissues.

A method of testing of the potency of the antibacterial activity of honey has been developed in the Honey Research Unit at the University of Waikato that compares the antibacterial potency of honeys with that of a standard antiseptic, phenol, when tested against a standard strain of *S. aureus* [54]. This is done both with and without catalase added so that the total activity and the non-peroxide activity can be measured.

So that consumers can easily compare the potency of the various brands of honey on sale, a Unique Manuka Factor (UMF) rating has been devised. This shows the concentration of phenol that has the same antibacterial potency as the non-peroxide activity in a sample of honey. The producers of active manuka honey have collectively registered UMF as a trademark, and have set a minimum level of UMF 10 (equivalent to 10 per cent w/v phenol) as a condition of this trademark being allowed to be used on a label.

Although the non-peroxide antibacterial component of manuka honey is stable when exposed to heat [60] and light (unpublished observations), when selecting honey for wound care the honey to be used would be better unpasteurised. It should also have been stored at a cool temperature protected from exposure to light, so that it has the maximum capacity for production of hydrogen peroxide as well. This is especially relevant since the hydrogen peroxide produced in honey may be of importance in the healing process beyond its action in clearing infection: hydrogen peroxide has been found to stimulate angiogenesis [68] and stimulate the growth of cultured fibroblasts [69].

Honey is usually pasteurised in processing to produce 'runny' honey that does not crystallise. Whilst such honey may be considered preferable for spreading on wound dressings, crystallised honey is more likely to have not been pasteurised. This can easily be made more fluid by warming to 37°C. Although raw honey may be perceived to carry a risk of infecting a wound, in none of the many reports published on the clinical usage of honey on open wounds was the honey that was used sterilised. However, there are no reports of any type of infection resulting from the application of honey to wounds [5].

If it is preferred that sterile honey is used, honey that has been treated by gamma-irradiation is available. This process kills clostridial spores in honey [70, 71] without loss of any of the antibacterial activity [70].

Conclusion

Honey is reported to give excellent results as a dressing for infected wounds. There are many reports of infection being cleared rapidly, but sometimes it is reported that infection is cleared more slowly.

This could be because of the large degree of variability in the potency of the antibacterial activity of honey. This is mostly due to enzymically generated hydrogen peroxide. However, there is a possibility that the hydrogen peroxide produced on a wound will be at least partly inactivated by the catalase activity in tissues and serum. If this is so, then honeys such as manuka honey or jellybush honey with a high level of non-peroxide antibacterial activity may prove to be more effective than other types of honey.

There is also the possibility that one type of antibacterial activity will penetrate better than the other into the depth of a wound and thus be more effective if infection is deeper than just on the wound surface. A comparative clinical trial with standardised honeys needs to be carried out to determine which type of honey is most effective. In the absence of comparative clinical data, rationally it would be best to select a honey with high levels of both types of antibacterial activity for use on infected wounds.

Because there is such a marked variation in honeys, the potency of antibacterial activity, even within a floral type, can only be established by laboratory testing of antibacterial activity.

References

1. Zumla A, Lulat A. Honey: a remedy rediscovered. J R Soc Med 1989; **82**:384–85.
2. Beck BF, Smedley D. Honey and your health (2nd ed). New York: McBride, 1944.
3. Forrest RD. Early history of wound treatment. J R Soc Med 1982; **75**:198–205.
4. Wadi M, Al-Amin H, Farouq A, Kashef H, Khaled SA. Sudanese bee honey in the treatment of suppurating wounds. Arab Medico 1987; **3**:16–18.
5. Molan PC. A brief review of honey as a clinical dressing. Primary Intention 1998; **6**(4):148–58.
6. Bose B. Honey or sugar in treatment of infected wounds? Lancet 1982; **i** (April 24):963.
7. Condon RE. Curious interaction of bugs and bees. Surgery 1993; **113**(2):234-35.
8. Green AE. Wound healing properties of honey. Br J Surg 1988; **75**(12):1278.
9. Keast-Butler J. Honey for necrotic malignant breast ulcers. Lancet 1980; **ii** (October 11):809.
10. Mossel DAA. Honey for necrotic breast ulcers. Lancet 1980; **ii** (November 15):1091.
11. Seymour FI, West KS. Honey: its role in medicine. Med Times 1951; **79**:104–07.
12. Somerfield SD. Honey and healing. J R Soc Med 1991; **84**(3):179.
13. Tovey FI. Honey and healing. J R Soc Med 1991; **84**(7):447.
14. Molan PC. The antibacterial activity of honey. 1. The nature of the antibacterial activity. Bee World 1992; **73**(1):5–28.
15. Dold H, Witzenhausen R. Ein Verfahren zur Beurteilung der örtlichen inhibitorischen (keimvermehrungshemmenden) Wirkung von Honigsorten verschiedener Herkunft. Z Hyg Infektionskr 1955; **141**:333–37.
16. Molan PC. The antibacterial activity of honey, 2. Variation in the potency of the antibacterial activity. Bee World 1992; **73**(2):59-76.
17. Soffer A. Chihuahuas and laetrile, chelation therapy,and honey from Boulder, Colo. Arch Intern Med 1976; **136**:865-6.
18. Gunther RT. The Greek herbal of Dioscorides. New York: Hafner, 1934 (Reprinted 1959).
19. Aristotle. Historia Animalium. Oxford, U.K: Oxford University, (350 BC) 1910.
20. Floris I, Prota R. Sul miele amaro di Sardegna. Apic Mod 1989; **80**(2):55-67.
21. Fotidar MR, Fotidar SN. 'Lotus' honey. Indian Bee J 1945; **7**:102.
22. Abbas T. Royal treat. Living in the Gulf, 1997:50-1.
23. Chirife J, Scarmato G, Herszage L. Scientific basis for use of granulated sugar in treatment of infected wounds. Lancet 1982; **i** (March 6):560-1.
24. Chirife J, Herszage L, Joseph A, Kohn ES. *In vitro* study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. Antimicrobial Agents and Chemotherapy 1983; **23**(5):766-73.
25. Herszage L, Montenegro JR, Joseph AL. Tratamiento de las heridas supuradas con azucar granulado comercial. Bol Trab Soc Argent Cir 1980; **41**(21-22):315-30.
26. Efem SEE. Clinical observations on the wound healing properties of honey. Br J Surg 1988; **75**:679-81.
27. Cavanagh D, Beazley J, Ostapowicz F. Radical operation for carcinoma of the vulva: a new approach to wound healing. J Obstet Gynaecol Br Commonw 1970; 77(11):1037-40.
28. Armon PJ. The use of honey in the treatment of infected wounds. Trop Doct 1980; **10**: 91.
29. Braniki FJ. Surgery in Western Kenya. Ann R Coll Surg Engl 1981; **63**:348-52.
30. Knutson RA, Merbitz LA, Creekmore MA, Snipes HG. Use of sugar and povidone-iodine to enhance wound healing: five years experience. South Med J 1981; **74**(11):1329-35.

31. Middleton K, Seal DV. Sugar as an aid to wound healing. Pharm J 1985; **235**:757-8.

32. Shimamoto Y, Shimamoto H, Fujihata H, Nakamura H, Matsuura Y. Topical application of sugar and povidone-iodine in the management of decubitus ulcers in aged patients. Hiroshima J Med Sci 1986; **35**(2):167-9.

33. Brånemark PI, Ekholm R, Albrektsson B, Lindstrsöm J, Lundborg G, Lundskog J. Tissue injury caused by wound disinfectants. J Bone Jt Surg Am Vol 1967; **49**(1):48-62.

34. Lineaweaver W, Howard R, Soucy D, McMorris S, Freeman J, Crain C, Robertson J, Rumley T. Topical antimicrobial toxicity. Arch Surg 1985; **120**(3):267-70.

35. Leistner L, Rödel W. The significance of water activity for microorganisms in meats. In: Duckworth RB (ed). Water Relations of Foods. London: Academic Press, 1975:309-23.

36. Willix DJ, Molan PC, Harfoot CJ. A comparison of the sensitivity of wound infecting species of bacteria to the antibacterial activity of manuka honey and other honey. J Appl Bacteriol 1992; **73**:388-94.

37. Postmes TJ, Bosch MMC, Dutrieux R, van Baare J, Hoekstra MJ. Speeding up the healing of burns with honey: an experimental study with histological assessment of wound biopsies. In: Mizrahi A, Lensky Y (eds). Bee Products: Properties, Applications and Apitherapy. New York: Plenum Press, 1997:27-37.

38. Hutton DJ. Treatment of pressure sores. Nurs Times 1966; **62**(46):1533-4.

39. Efem SEE. Recent advances in the management of Fournier's gangrene: preliminary observations. Surgery 1993; **113**(2):200-4.

40. Phuapradit W, Saropala N. Topical application of honey in treatment of abdominal wound disruption. Aust N Z J Obstet Gynaecol 1992; **32**(4):381-4.

41. Harris S. Honey for the treatment of superficial wounds: a case report and review. Primary Intention 1994; **2**(4):18-23.

42. Ndayisaba G, Bazira L, Habonimana E, Muteganya D. Clinical and bacteriological results in wounds treated with honey. J Orthop Surg 1993; **7**(2):202-4.

43. Subrahmanyam M. A prospective randomised clinical and histological study of superficial burn wound healing with honey and silver sulfadiazine. Burns 1998; **24**(2):157-61.

44. Dumronglert E. A follow-up study of chronic wound healing dressing with pure natural honey. J Nat Res Counc Thail 1983; **15**(2):39-66.

45. Vardi A, Barzilay Z, Linder N, Cohen HA, Paret G, Barzilai A. Local application of honey for treatment of neonatal postoperative wound infection. Acta Paediatr 1998; **87**(4):429-32.

46. Smith M, Enquist IF. A quantitative study of impaired healing resulting from infection. Surg Gynecol Obstet 1967; **125**(5):965-73.

47. Wood B, Rademaker M, Molan PC. Manuka honey, a low cost leg ulcer dressing. N Z Med J 1997; **110**:107.

48. Postmes T, van den Bogaard AE, Hazen M. Honey for wounds, ulcers, and skin graft preservation. Lancet 1993; **341**(8847):756-7.

49. Greenwood D. Honey for superficial wounds and ulcers. Lancet 1993; **341**(8837):90-1.

50. d'Agostino Barbaro A, La Rosa C, Zanelli C. Atttività antibatterica di mieli siciliani. Quad Nutr 1961; **21**(1/2):30-44.

51. Dustmann JH. Antibacterial effect of honey. Apiacta 1979; **14**(1):7-11.

52. Buchner R. Vergleichende Untersuchungen über die antibakteriellen Wirkung von Blüten und Honigtauhonigen. Südwestdeutscher Imker 1966; **18**:240-1.

53. Christov G, Mladenov S. Propriétés antimicrobiennes du miel. C R Acad bulg Sci 1961; **14**(3):303-6.

54. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 1991; **43**(12):817-22.

55. White JW, Subers MH, Schepartz AI. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim Biophys Acta 1963; **73**:57-70.

56. White JW, Subers MH. Studies on honey Inhibine, 3. Effect of heat. J Apic Res 1964; **3**(1):45-50.

57. White JW, Subers MH. Studies on honey Inhibine, 4. Destruction of the peroxide accumulation system by light. J Food Sci 1964; **29**(6):819-28.

58. Adcock D. The effect of catalase on the inhibine and peroxide values of various honeys. J Apic Res 1962; **1**:38-40.

59. Bogdanov S. Characterisation of antibacterial substances in honey. Lebensm Wiss Technol 1984; **17**(2):74-6.

60. Molan PC, Russell KM. Non-peroxide antibacterial activity in some New Zealand honeys. J Apic Res 1988; **27**:62-7.

61. Roth LA, Kwan S, Sporns P. Use of a disc-assay system to detect oxytetracycline residues in honey. J Food Prot 1986; **49**(6):436-41.

62. Dille RS, Watkins CH. A method for the determination of plasma catalase and the values obtained in normal adults. J Lab Clin Med 1948; **33**:480-6.

63. White JW, Subers MH. Studies on honey Inhibine, 2. A chemical assay. J Apic Res 1963; **2**(2):93-100.

64. Cohen G, Hochstein P. Glutathione peroxidase: the major pathway for the detoxification of hydrogen peroxide in erythrocytes. American Chemical Society. 141st. Meeting. Division of Biological Chemistry, 1962.

65. Cooper RA, Molan PC. The use of honey as an antiseptic in managing *Pseudomonas* infection. J Wound Care 1999; **8**(4):161-4.

66. Molan P, Brett M. Honey has potential as a dressing for wounds infected with MRSA. The Second Australian Wound Management Association Conference; 1998 March 18-21; Brisbane, Australia.

67. Allen KL, Molan PC, Reid GM. The variability of the antibacterial activity of honey. Apiacta 1991; **26**(4):114-21.

68. Tur E, Bolton L, Constantine BE. Topical hydrogen peroxide treatment of ischemic ulcers in the guinea pig: blood recruitment in multiple skin sites. J Am Acad Dermatol 1995; **33**(2 Pt 1):217-21.

69. Schmidt RJ, Chung LY, Andrews AM, Turner TD. Hydrogen peroxide is a murine (L929) fibroblast cell proliferant at micro to nanomolar concentrations. Second European Conference on Advances in Wound Management; 1992 October 20-23; Harrogate, UK.

70. Molan PC, Allen KL. The effect of gamma-irradiation on the antibacterial activity of honey. J Pharm Pharmacol 1996; **48**:1206-9.

71. Postmes T, van den Bogaard AE, Hazen M. The sterilisation of honey with cobalt 60 gamma radiation: a study of honey spiked with *Clostridium botulinum* and *Bacillus subtilis*. Experentia (Basel) 1995; **51**:986-9.