

Antibacterial Properties of Manuka Honey and the Role of Methylglyoxal

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ABSTRACT

The unique ecosystems of New Zealand have produced a diverse range of honey over the years, with Manuka honey being one of the most renowned. Produced by Western honeybees extracting nectar from Manuka flowers, this monofloral honey has become known for its distinct antibacterial and anti-inflammatory properties. Whilst antibacterial activity in other honey tend to stem from factors such as hydrogen peroxide content, high viscosity, osmotic effect, and acidic pH, the antibacterial activity of Manuka honey is mainly attributed to methylglyoxal (MGO), a dicarbonyl compound which is found in high concentrations in Manuka honey. This review paper will focus on the antibacterial properties of Manuka honey and the role that MGO plays. Understanding the specific chemical mechanisms that of attack on different strains of bacteria by Manuka honey and the role of MGO is crucial to potentially understanding how new drugs or medicines can combat antibacterial resistance to antibiotics.

The Origin of Manuka Honey

The ecosystems of the North and South Islands of New Zealand (NZ) have naturally developed a large variety of unique honey samples (1). Among the diverse variety of honey in NZ is Manuka (*Leptospermum scoparium*), known for its antibacterial activity and the ability to repair wounds (2). Manuka honey is a monofloral honey which is produced by Western honeybees (*Apis mellifera*) through extracting nectar from Manuka flowers (3).

Peter Molan and his team at University of Waikato showed that Manuka honey can resist bacterial *Staphylococcus aureus* (*S.aureus*) on a culture plate. It is much more potent than any other honey samples. Remarkably, Manuka honey even retained its antibacterial properties at low concentration and high temperatures (95 °C) (1). Some published research papers report tests of Manuka honey on other strains of bacteria such as *Pseudomonas aeruginosa* (*P.aeruginosa*) and *Escherichia coli* (*E. coli*) and proposed that the suppression of the growth of different species of bacteria by Manuka honey utilizes different mechanisms (4).

Unique Properties of Manuka Honey

For many honeys, their antibacterial activity stems from hydrogen peroxide content. However, Manuka honey exhibits a significant amount of non-peroxide antibacterial activity which is largely due to the presence of MGO (5,6). MGO itself is a molecule that is found in a range of other foodstuffs and beverages, such as wine, bread, and dairy products (7-9). However, Manuka honey is unique in that upon testing samples of Manuka honey, researchers have reported the presence of large amounts of methylglyoxal in each sample which correlated with its non-peroxide activity (6). It is also well known among NZ beekeepers that MGO content and hence non-peroxide antibacterial activity increase over time with storage (10)

Manuka honey can also be used to treat wounds. Manuka honey creates a high viscosity protective barrier on the wound and prevents bacterial infection (1). Manuka honey's hygroscopicity also contributes to wound healing as it absorbs and holds the moisture around the wound area, decreasing the chance of bacterial survival (11). In addition,

it also has the potential to minimize hypertrophic scarring. The pH of Manuka Honey generally ranges from 3.5 to 4.5 which can increase the oxygen release from haemoglobin around the wound area. This excess oxygen activates fibroblasts which help to further stimulate wound healing (12).

One of the key issues in wound healing recovery is bacterial infection and subsequently inflammation occurred (13). Manuka honey has been shown to be beneficial in wound healing, primarily due to its antibacterial activity. In addition, it is also known to have Leptosperin which is an anti-inflammatory agent that enables it to decrease wound inflammation effectively (14). Manuka honey can also stimulate angiogenesis, epithelialization, and granulation to speed up the rate of healing and proliferate fibroblasts. A combination of Manuka honey's anti-inflammatory process and stimulatory effects on epithelialization and granulation help to rapidly reduce pain and edema (12).

Interestingly, Manuka honey failed to gain notoriety early on due to harvesting issues; Manuka honey was much more difficult to extract than other alternatives. Additionally, Manuka honey did not seem to have any outstanding properties that made it worth extracting (1). However, this changed when Molan published a paper confirming the unique antibacterial effects of Manuka honey, specifically emphasizing the use of honey as a wound dressing which attracted huge media attention across the world (15). Since then, the demand for Manuka honey increased significantly, and eventually led to the creation of the Unique Manuka Factor (UMF), which reflects the level of MGO concentration in a sample of honey (16) and sets apart authentic Manuka honey from other blended, multifloral honeys (1). Grades of UMF 24+ are classified as Superior Rare High Grade, UMF 15+ to 20+ Ultra-Premium Grade, UMF 10+ to 15+ Premium Grade, UMF 5+ to 9+ Certified Authentic. The higher the grade, the greater the antibacterial activity and the more severe a wound the honey can treat (17). Manuka honey can easily be distinguished since it contains MGO, a natural molecule responsible for the antibacterial properties of the honey, and DHA (dihydroxyacetone), the precursor of MGO that slowly converts to MGO over time and hence dictates the amount of MGO content in a jar of honey and its shelf life (18).

Where MGO in Manuka honey comes from

MGO is derived from its parent molecule dihydroxyacetone (DHA) and is converted from DHA to MGO through non-enzymatic Maillard reactions (4). The conversion from DHA to MGO is essentially a non-reversible dehydration reaction which requires both acidic and basic conditions (19). If conditions are only acidic aqueous, then only dehydration of DHA to MGO occurs. On the other hand, basic conditions would result in isomerization from DHA to glyceraldehyde. Thus, both isomerization and dehydration would occur under a buffer solution such as phosphate or acetate, which contain both acid-base catalysts (20) (Figure 1).

However, another study suggests that both DHA and Glyceraldehyde form a common intermediate enediol compound first, with the enediol compound being dehydrated instead of the DHA or Glyceraldehyde (21) (Figure 2). This mechanism is significant during the storage of Manuka honey since MGO levels within the honey tend to rise over time due to conversion from DHA to MGO (4).

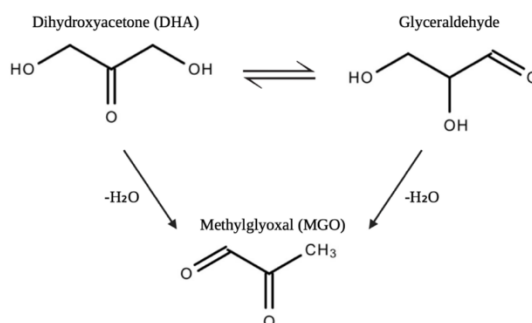


Figure 1. Conversion of DHA to Glyceraldehyde and MGO.

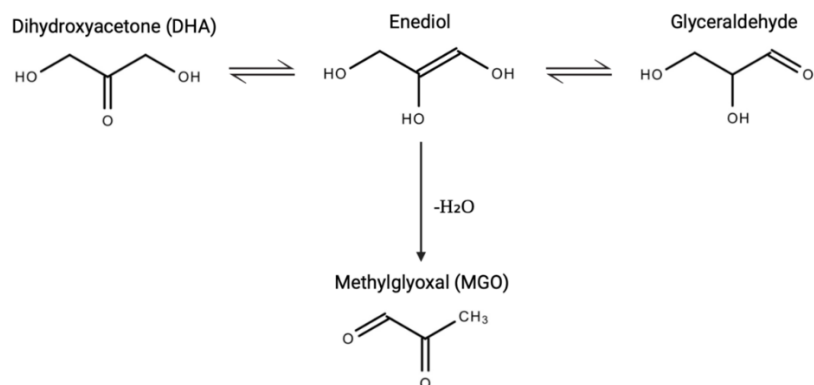


Figure 2. Conversion of DHA to Glyceraldehyde and MGO with Enediol intermediate.

Upon adding MGO to a sample of clover honey, Adams et al. (10) found that there was little change in concentration of MGO and practically no formation of DHA, which further suggested the irreversibility of the proposed mechanism of the conversion from DHA to MGO by dehydration mechanism. However, addition of DHA to clover honey stored at 37 degrees Celsius showed the formation of MGO and decrease in DHA over time. Particularly, the higher the concentration of DHA, the higher the rate of formation of MGO. They also demonstrated that the conversion from DHA to MGO did not produce a 1:1 ratio and hence was not balanced in terms of stoichiometry (22), suggesting that DHA and MGO also took part in side-reactions with other components in Manuka honey (23).

The presence of amino acids in Manuka honey such as lysine and arginine also catalyze the conversion of DHA to MGO (10). Other studies suggest that DHA potentially undergoes reactions with amino acids, since DHA is more reactive than glucose on reaction with amino acids and has been found to be especially reactive with amino acids of relatively higher pH such as lysine and proline. (24).

MGO in Manuka honey plays a critical role in antibacterial activity

Manuka honey's non-peroxide antibacterial activity can be mainly attributed to MGO. The antibacterial activity of MGO is mainly due to its ability to inactivate proteins through cross-linking them (25). Hydrogen peroxide is also present in Manuka honey, although in relatively lower concentrations compared with non-Manuka honeys. Nevertheless, hydrogen peroxide still plays a role in Manuka honey through interacting with other components in Manuka honey and producing hydroxyl radicals to increase overall antibacterial activity (26).

A study by Weigel et al. (27) showed evidence of 1,2-dicarbonyl compounds in honey, specifically 3-deoxyglucosulose (3-DG), methylglyoxal (MGO), and glyoxal (GO). Mavric et al. (6) tested the inhibiting effects of these three 1,2-dicarbonyl compounds found in Manuka Honey on two different strains of bacteria, *E. coli* and *S. aureus*, finding that MGO had the most pronounced antibacterial effect out of all three compounds at a minimum inhibitory concentration (MIC) of 1.1mM for both strains of bacteria (Table 1). The researchers were also able to verify that MGO was the main contributor to antibacterial activity using an Agar well diffusion assay with *S. aureus* as the bacteria. Testing equally concentrated samples of forest honey and Manuka honey, the sample of Manuka honey showed a clear inhibition zone while the sample of forest honey did not. MGO was confirmed to be the key contributor of antibacterial activity when it was found that the inhibition zone of the MGO-supplemented forest honey was similar to that of the original Manuka honey sample when an equivalent amount of MGO found in the sample of Manuka honey was added to another sample of forest honey.

Table 1. Minimum inhibitory concentration (MIC) of solutions of 1,2-dicarbonyl compounds adapted from Mavric et al (6)

Sample	MIC for <i>E. coli</i> (mM)	MIC for <i>S. aureus</i> (mM)
3-DG	No inhibition	No inhibition
GO	6.9	4.3
MGO	1.1	1.1

Changes to antibacterial activity due to MGO-infusion into honey

Jervis et al. (28) wanted to establish the effects of adding additional amounts of MGO to Manuka and non-Manuka honey on biofilms containing *S. aureus* bacterial strains. Addition of Manuka Honey to biofilms showed biocidal activity at higher concentrations of 66.00% and 33.00% w/v (Table 2), and when only MGO was added to biofilms, biocidal activity was present at concentrations of 1.05 mg/ml and higher, while any concentration lower showed lack of biocidal activity (Table 3). On the other hand, non-MGO honeys did not demonstrate any form of biocidal activity at any concentration, yet when non-MGO honey was infused with MGO, the infused samples showed almost equivalent biocidal activity as Manuka honey with 790mg/kg MGO (Table 2). This not only proved that antibacterial activity could be increased through infusion of MGO, but it also further reinforced the significant role of MGO in Manuka honey (6).

Table 2. Biocidal activity of various honeys at differing concentrations adapted from Jervis et al. (28)

% Honey Concentration (w/v)	CH only	MH (790mg/kg MGO) only	CH = 790mg/kg MGO	MH 2370mg/kg MGO +	CH 3160mg/kg MGO +
66.00	NB	B	B	B	B
33.00	NB	B	B	B	B
16.50	NB	NB	NB	B	B
8.25	NB	NB	NB	NB	NB

Cell color in this table corresponds to equivalent MGO-only concentration in Table 3.

Key: CH = capilano/non-MGO honey; MH = Manuka honey; MGO = methylglyoxal; NB = not biocidal; B = biocidal

Table 3. Biocidal activity of MGO-only Solutions adapted from Jervis et al. (28)

MGO Concentration (mg/ml)	Biocidal activity
2.11	B
1.05	B
0.53	NB
0.26	NB
0.13	NB
0.06	NB
<0.01	NB

Key: NB = not biocidal; B = biocidal

The different mechanisms of the antibacterial activity of MGO against bacteria

Manuka honey has been shown to be effective against biofilm forms of *P. aeruginosa* and *S. aureus*. (29). One study (30) tested MGO against these two strains of bacteria, finding that MGO was an effective agent against *S. aureus* and *P. aeruginosa*. However, they were unable to identify the mechanisms of actions of MGO against *S. aureus* and *P. aeruginosa*. Jenkins et al. (4) have suggested that MGO inhibits different bacteria strains via different mechanisms. This view was supported by other studies that proposed that MGO inhibited *P. aeruginosa* via cell lysis while *S. aureus* was inhibited through cell division. (31,32)

Proposed mechanism of MGO on *S. aureus*

S. aureus typically duplicates its cells through segregating its chromosome to form two daughter cells that are still connected. The daughter cells are fully separated when murein hydrolase degrades the cell wall between the two cells (33). The mechanism proposed below shows how the *S. aureus* cell can achieve all the steps including septa completion. Yet, MGO prevents the final step of two daughter cells separating by inhibiting the activity of murein hydrolase and hence causing the formation of many septated but non-dividing *S. aureus* cells. It's interesting to note that many papers classify this mechanism against *S. aureus* as bactericidal (32, 34, 35), yet the proposed mechanism by Jenkins et al. (4) leans more towards bacteriostatic activity.

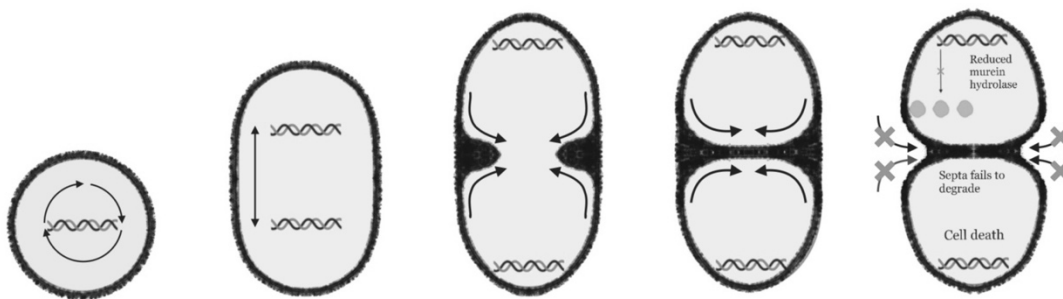


Figure 3. Representation of the mechanism by which MGO inhibits a *S. aureus* cell adapted from Jenkins et al. (4)

Proposed mechanism of MGO on *P. aeruginosa*

On the other hand, the mechanism by which MGO attacks *P. aeruginosa* cells is completely different to *S. aureus*, with *P. aeruginosa* cells undergoing cell lysis and losing its structural integrity when inhibitory concentrations of Manuka Honey and MGO are present (31). The structural integrity of *P. aeruginosa* cells is dependent on protein F (OprF) (36), which ensures that there is a link between the outer membrane layer and the peptidoglycan layer underneath to keep a regular cell shape (37). However, when MGO is added, a decrease in OprF leads to the loss of covalent cross links between the membrane and peptidoglycan layer, causing the membrane to become subject to osmotic stress, and eventually leading to membrane blebbing and cell lysis (4).

Roberts et al. (38) suggests that MGO reduces the expression for four of the flagellum-associated genes (*fliA*, *fliC*, *fleN* and *fleR*) and hence decreases the number of flagellated cells. Reduced flagellation means reduced motility and increased retarded movement of the *P. aeruginosa* cells, meaning that *P. aeruginosa* cells are less likely to disseminate into the bloodstream (4).

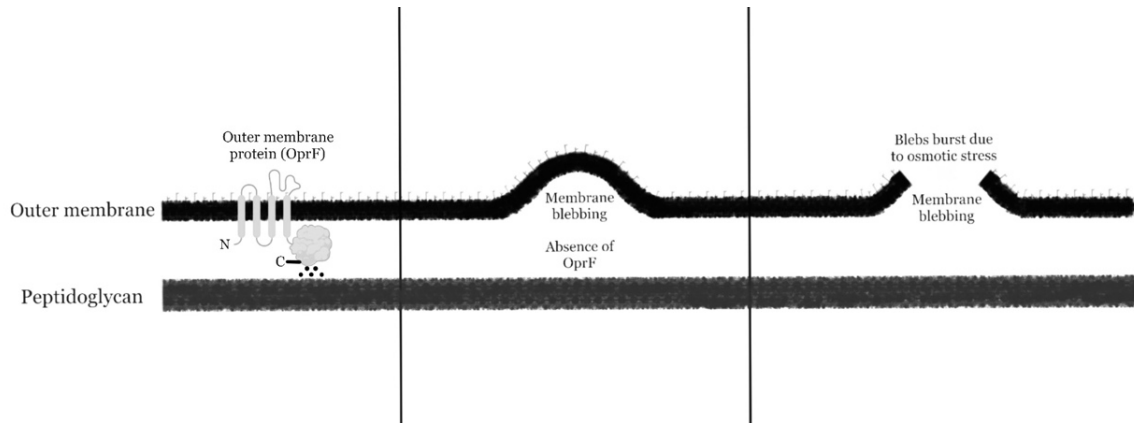


Figure 4. Representation of the mechanism by which MGO inhibits a *P. aeruginosa* cell adapted from Jenkins et al. (4)

Proposed mechanism on *E. coli*

Aside from *S. aureus* and *P. aeruginosa*, Manuka honey and MGO could also affect *e. coli*'s cellular membrane permeability, which is closely linked with the ability of a cell to control its metabolism and energy (39). After application of MGO to a biofilm of *E. coli*, the quantities of potassium ions and proteins leaked from these cells increased over time, showing how MGO disrupted the cell membrane and suggesting that *E. coli* cells had undergone cell lysis and ultimately died (40).

Other factors in Manuka honey that contribute to antibacterial activity

Osmotic effect:

Manuka honey is essentially a super-saturated solution containing monosaccharides glucose and fructose, with water content making up only a small proportion of its weight (41). Since these monosaccharides interact with most water molecules through hydrogen bonding, there are very few free water molecules left for bacteria to thrive in (42). In other words, the level of *Water activity* (a_w) is too low to support the growth of many different strains of bacteria. Yet there are still some bacteria which can withstand low a_w . For example, *S. aureus* is very osmotolerant due to its ability to accumulate glycine, betaine and proline, (43), meaning that Manuka honey can't inhibit its bacterial growth.

Hydrogen peroxide:

Another contributing factor to Manuka Honey's antibacterial activity is H_2O_2 , which produces hydroxyl free radicals that can cause cell death, tissue injury, and damage DNA (44, 45). The mechanism below (Figure 5) shows how glucose, on reaction with oxygen, water, and glucose oxidase enzymes, produces gluconic acid and H_2O_2 . Thus, the amount of H_2O_2 content in a sample of Manuka Honey is closely related to the acidic pH of the honey, which is primarily attributed to the presence of gluconic acid (42). The production of gluconic acid lowers the pH to levels that cause the enzyme activity of glucose oxidase to drop to almost zero, making glucose oxidase enzymes rather inactive and the level of H_2O_2 to be almost negligible in Manuka honey (46). Nevertheless, H_2O_2 still contributes to the antibacterial activity of Manuka Honey since H_2O_2 is produced at a continuous rate, which is more effective than when added in bulk as a bolus (47).

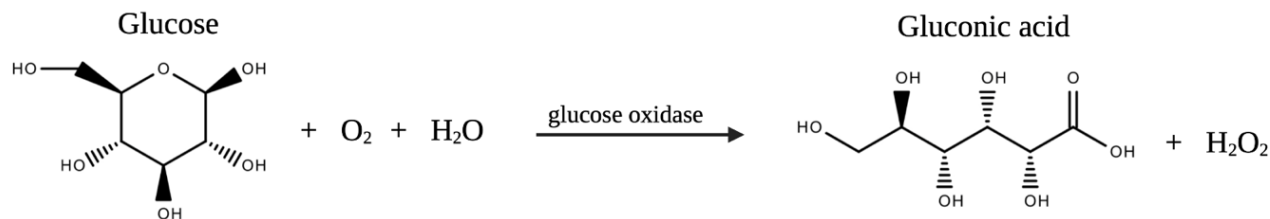


Figure 5. The glucose oxidase reaction that produces H_2O_2 .

Manuka honey and antibiotics

There is also clinical evidence to show that Manuka honey can augment the action of modern antibiotics. Some of these antibiotics include Rifampicin, which is a clinically used antibiotic for treatment of tuberculosis (48), while Oxacillin is often used to treat staphylococcal infections. (49) On the other hand, Tetracycline is an effective antibiotic for the treatment of acne (50), and Imipenem can be used to treat other severe skin infections (51).

Jenkins et al. (52) provided evidence for a few of these antibiotics being synergistic with Manuka honey. The two different strains of bacteria tested, *P. aeruginosa* and methicillin resistant *S. aureus* (MRSA), showed higher susceptibility to a combination of Tetracycline and Manuka honey, than just Tetracycline itself. Other antibiotics were also tested as well: Rifampicin and Imipenem. Imipenem and Manuka honey were found to be synergistic towards MRSA but not *P.aeruginosa*, while Rifampicin and Manuka honey were synergistic towards *P. aeruginosa*. Manuka honey also did not enhance the activity of Rifampicin against MRSA.

However, Liu et al. (53) had contradictory results, finding that MRSA strains became more sensitive to a combination of Rifampicin and Manuka honey. This nuance in results may be potentially attributed to the Manuka honey sources used by the two different studies, with Liu et al. (53) using unprocessed Manuka honey from Hokianga, NZ, while Jenkins et al. (52) using Manuka honey purchased from Oxoid in Cambridge, United Kingdom. Nevertheless, Liu et al. stated that Rifampicin and Manuka honey showed additive effects, further reinforcing the conclusion made by Jenkins et al. (52) that this combination was not synergistic towards *S. aureus*.

MGO and Rifampicin:

Muller et al. (54) also examined the synergistic effects of MGO and Rifampicin to see if MGO was solely responsible for the Manuka honey-Rifampicin synergistic effect. They found that MGO was not completely synergistic with Rifampicin (unlike the Manuka honey and Rifampicin combination), since growth of *S. aureus* strains weren't completely inhibited. The reason for this was that MGO with Rifampicin was only an additive and not synergistic combination.

However, when Mukherjee et al. (55) tested a similar combination of MGO and Rifampicin on *P. aeruginosa* strains instead of *S. aureus* strains, MGO was found to be synergistic with Rifampicin. These two different studies potentially suggest that MGO becomes synergistic with Rifampicin depending on the bacteria strain, however, further research and experiments would be needed to verify this.

Limitations

Risks of antibacterial resistance associated with Manuka honey:

Jervis et al. (28) suggest that for clinical purposes, a combination of MGO and honey solution would yield stronger biocidal activity than that of an MGO-only solution, since the antibacterial activity of MGO is enhanced when it is in the presence of other components found in Manuka honey. Cooper et al. (56) also suggest that extracting specific active components of honey such as MGO and using it alone on bacteria would only increase the chances of bacteria developing resistance to the antibacterial effects of Manuka honey.

Camplin et al. (57) reported that isolates of *P. aeruginosa* from Manuka honey treated biofilms exhibited greater minimum inhibitory concentrations (MICs) than their progenitor strains, whose biofilms weren't treated with Manuka honey, when antibiotics Rifampicin and Imipenem were added separately. Even after repetition of the experiment, the Manuka honey treated isolates still exhibited increased resistance.

Relationship of concentration levels of Manuka honey to bacterial susceptibility to honey:

Cooper et al. (56) showed that increasing the concentration of Manuka honey stepwise over long-term would decrease the abilities of *S. aureus* and *P. aeruginosa* strains to develop Manuka honey resistance. Nevertheless, the acquisition of antibacterial resistance still couldn't be prevented through this method.

Additionally, Liu et al. (53) also tested a combination of Oxacillin and Manuka honey, finding that the combination helped to restore oxacillin susceptibility to a MRSA strain, which was similar to the conclusion drawn by Jenkins et al. (58). Muller et al. (54) also showed that Medihoney (a medical product containing very high concentrations of Manuka honey) combined with Rifampicin was unable to restore susceptibility to Rifampicin for Rifampicin-resistant *S. aureus* strains, illustrating that Manuka honey doesn't necessarily restore antibiotic susceptibility for all different species of bacteria.

The possible side effects of Manuka honey:

Aron et al. (59) tested the effects of Manuka honey on treating ear infections of chinchillas. On analysis of the cochleae applied with Manuka honey, they found that the honey had bactericidal properties on biofilms of *P. aeruginosa* and *S. aureus* but the concentration used also led to facial paralysis and hearing loss for the chinchilla.

In the treatment of diabetic ulcers, MGO reacts with protein residues such as lysine and arginine, leading to the formation of advanced glycation end products (AGEs) (60). Increased AGEs accumulation is often associated with diabetic lesions and enhances the activity of neutrophils which leads to long term chronic inflammation (61,62).

Conclusion

Although much is already known about the antibacterial properties of Manuka honey, there still needs to be further research to solidify the mechanisms by which MGO and other compounds in Manuka honey exert on different species of bacteria. Additionally, more research on different strains of bacteria is needed, as a large majority of published research has mainly focused on only three strains: *S. aureus*, *P. aeruginosa*, and *E. coli*. As researchers gain a greater understanding of how the components in Manuka honey work together, there is also a need for investigating the effects of combining the use of Manuka honey with conventional antibiotics to combat the inevitable development of antibacterial resistance.

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