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Revolutionizing non-conventional wound healing using honey by simultaneously targeting multiple molecular mechanisms

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ABSTRACT

Hospital-acquired infections and treatment-related wound complications constitute a tremendous burden for the health care system, particularly given the serious increase in multidrug resistant pathogens. Imagine that a large part of nosocomial infections can be prevented using a simple treatment. In this respect, honey is used mainly in topical cutaneous wound care because of its potent broad-spectrum antibacterial and wound healing activities. However, therapeutic use outside this scope has been limited. The current review provides an in-depth view of studies using honey outside the conventional wound care indications. Non-conventional routes of honey application include subcutaneous, intra-socket, abdominal, and oral administration in novel indications, such as post colon surgery, mucositis, and tooth extraction. Honey consistently demonstrates beneficial therapeutic activities in these novel applications, orchestrating antimicrobial and prophylactic activity, reducing inflammation and wound dehiscence, and inducing healing, epithelialization, and analgesic activity. Several molecular mechanisms are responsible for these beneficial clinical effects of honey during the course of wound healing. Pro-inflammatory effects of honey, such as induction of iNOS, IL-1β, and COX-2, are mediated by TLR4 signaling. In contrast, honey's anti-inflammatory actions and flavonoids induce anti-inflammatory and antioxidant pathways by inducing NRF2 target genes, including HO-1 and PRDX1. The molecular and biochemical pathways activated by honey during the different phases of wound healing are also discussed in more detail in this review. Variation between different honey origins exists, and therefore standardized medical-grade honey may offer an optimized and safe treatment. Honey is a valuable alternative to conventional antimicrobial and anti-inflammatory therapies that can strongly reduce nosocomial infections.

Treatment-related infections continue to contribute to morbidity and mortality; multi-resistant pathogens are on the rise

Getting a baby was a risky business in the 19th century. Remarkably, the odds of getting childbirth fever and dying of sepsis as a mother was 20 times higher when treated by a physician instead of a midwife. Physicians, first doing autopsies on mothers that had deceased, were next performing childbirth. Semmelweis demonstrated that post-partem infections "childbed fever", and subsequently the death of these mothers significantly decreased when these physicians simply washed their hands with calcium hypochlorite before obstetric procedures. Although this reduced mortality from 20% to 2%, the conservative physicians could not believe that they were the cause of death. Only when the "germ theory" of Pasteur became known and Koch linked *Bacillus anthracis* to anthrax, the theories of Semmelweis were proven to be correct, and he was posthumously praised (Noakes et al., 2008).

Nowadays, hospital-acquired (nosocomial) infections, such as surgical site infections (SSI), still form an enormous burden on human

Abbreviations: TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; HO-1, heme oxygenase-1; SOD, superoxide dismutase; GSH, glutathione; VEGF, vascular endothelial growth factor; Ang1/2, angiopoietin-1/2; nrf-2, nuclear factor erythroid 2-related factor 2; PRDX1, peroxiredoxin 1; α-SMA, α-smooth muscle actin; MMPs, matrix metalloproteinases.

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medicine. Despite the use of antibiotics and other preventive efforts, the morbidity of SSIs is still about 2–5% of all surgical procedures, and financial costs for treating these infections are rising over the years (Berrios-Torres et al., 2017; de Lissovoy et al., 2009; Tun et al., 2018). Moreover, micro-organisms rapidly become resistant to antibiotics, making it increasingly challenging to treat SSI with conventional treatments (Cheadle, 2006). There is thus an urgent need for both prevention and effective treatment (Curtis, 2008).

Honey exerts antimicrobial and healing effects and is already an approved topical method to aid skin wound healing (Molan, 2006). In contrast to traditional antimicrobial drugs, no resistance to honey has been reported (Cooper and Jenkins, 2009; Nolan et al., 2020). This likely relates to the multiple mechanisms of antimicrobial activity, making it hard for pathogens to develop resistance (Nolan et al., 2019, 2020) This is an important feature since an increasing fraction of pathogenic bacteria turn into 'superbugs' by developing multidrug resistance to antibiotics, as reflected by the increased number of hospital-acquired infections with resistant bacteria (Gashaw et al., 2018; Morris and Cerceo, 2020). In parallel with Semmelweis' "washing hands", administration of honey to surgical wounds may greatly impact the risk of developing nosocomial infections while being inexpensive and straightforward. The salutary properties of honey for wound care are based on two main principles: its antimicrobial and its pro-healing activity. Can honey revolutionize wound repair by preventing and treating severe infections and aiding the healing process? This review will present recent advances in the non-conventional use of honey for indications other than topical cutaneous wound healing, including intra-oral, intra-abdominal, and subcutaneous use in primarily closed wounds. Finally, we present the molecular and biochemical signaling pathways underlying some of the observed protective effects of honey.

Honey application protects against pathogens and improves wound repair

When applied to wounds, the high osmolarity of honey, due to the high sugar content, causes a hygroscopic effect at the wound site, retracting water from colonizing bacteria (Molan, 2006). This effect, in addition to the intrinsic low pH of honey, and the presence of antimicrobial molecules, creates an unsuitable environment for the invasion and survival of bacteria (Bang et al., 2003).

Two different types of honey exist, depending on the species of flowers that the bees pollinate (Molan, 2006; Nolan et al., 2019). The types can be distinguished based on their main antimicrobial mode of action: 'peroxide' versus 'non-peroxide'. The activity of the first type, the 'peroxide-based' honey group, is related to glucose oxidase, an enzyme secreted by the bee that, in the presence of water, converts the glucose in honey into gluconic acid and hydrogen peroxide (H_2O_2). The latter is bactericidal, even at the low concentrations generated in the honey, and is regarded as one of its main antimicrobial mechanisms (Nolan et al., 2020). The peroxide production is higher when the honey has a lower sugar concentration because of the higher enzyme activity of glucose oxidase in the presence of water (Bang et al., 2003). When honey is applied to the wound bed, retraction of water from the wound due to the high osmolarity of the honey automatically aids the process of hydrogen peroxide release. An important detail is that this release is spread in time, preventing accumulation and cytotoxic concentrations of hydrogen peroxide (Bang et al., 2003).

The main antimicrobial action of the second type, the 'non-peroxide-based' honey group, is related to the antimicrobial molecule methylglyoxal (MGO) (Gethin et al., 2008; Mandal and Mandal, 2011). Manuka honey is the best-known non-peroxide-based honey. The nectar of the flowers of the Manuka tree (*Leptospermum scoparium*) contains high amounts of dihydroxyacetone and so does the derived honey. The dihydroxyacetone is converted non-enzymatically by the Maillard reaction into MGO (Nolan et al., 2019). It is believed that the presence of MGO in the honey inhibits the glucose peroxidase enzyme as no hydrogen peroxide is produced (Majtan et al., 2013). Both honey types contain additional molecules that exert direct antimicrobial effects, including polyphenolic compounds (phenolic acids, flavonoids, and tannins) and antimicrobial peptides such as bee defensin-1 (Alvarez-Suarez et al., 2014; Gethin et al., 2008; Kwakman et al., 2010; Mavric et al., 2008).

Since honey exerts such a broad-spectrum of antimicrobial activities, they form ideal candidate drugs to prevent and treat bacterial infections (Nolan et al., 2020). Of note, recent studies demonstrated that honey also shows activity against other pathogens like fungi and viruses such as *Candida albicans, Candida auris*, herpes simplex virus, and varicella-zoster virus (de Groot et al., 2021; Hashemipour et al., 2014; Hermanns et al., 2019; Naik et al., 2021; Shahzad and Cohrs, 2012), implying more extensive properties. Moreover, honey can also eradicate biofilms, which are notorious for being persistent and hard to treat with antibiotics (Majtan et al., 2020; Pleeging et al., 2020).

Besides its antimicrobial properties, honey also has multiple physicochemical and molecular properties that enhance wound healing. Wound healing is a dynamic and a precisely coordinated process of sequential cellular, molecular, and biochemical events aimed to restore the integrity of the injured tissue as quickly as possible (Diegelmann and Evans, 2004). Following hemostasis, the healing process is divided into three distinct but overlapping phases: inflammation, cell proliferation, and remodeling (Fig. 1) (Broughton et al., 2006; Diegelmann and Evans, 2004).

Many cases in the clinic support honey playing a role in all phases of wound healing. The physicochemical properties of honey that stimulate wound healing include its osmotic activity and the creation of moist and an acidified wound microenvironment. This leads to an outflow of lymph fluid, promotes autolytic debridement, enriches circulation with a better supply of oxygen and nutrients, and creates an optimized environment for regenerating tissue (Molan, 1999). Honey forms a good nutrient source for the skin cells, such as fibroblasts during cell proliferation and keratinocyte migration during re-epithelialization. It is also widely reported that the formation of new blood vessels, angiogenesis, is accelerated by honey (Nisbet et al., 2010; Scepankova et al., 2021). Skin wounds treated with honey showed more fibroblastic, angiogenic, and epithelialization activity leading to faster granulation and closure of the wounds when compared to control groups having wounds treated with silver sulfadiazine (antibiotic effect), glucose (osmotic effect), or saline-soaked gauzes (moistening effect) (Molan, 2006). Honey-treated wounds also show less edema and inflammation (Molan, 2006). Dermal fibroblasts had improved viability, were more proliferative, and showed more migration in vitro (Ebadi and Fazeli, 2021; Nordin et al., 2018). Hence, phenolic constituents of honey act as antioxidants, scavenging free radicals created by activated neutrophils and macrophages, thus protecting the wound microenvironment (van den Berg et al., 2008). Clinical studies also demonstrate that honey can minimize scar formation, likely due to its anti-inflammatory, anti-oxidative, and remodeling properties. Finally, anti-nociceptive and analgesic effects are reported when wounds are treated with honey, making it a potential aid in post-surgical pain relief (Zakaria et al., 2015).

Due to the multiple mechanisms of action, the potential of honey in wound care is not limited to topical cutaneous wound repair. It can also be applied subcutaneously, intra-abdominally, and orally to aid wound repair (Fig. 2). Shown below is a compelling overview of the existing literature using honey in non-conventional applications in both humans and animals.

Improved repair of iatrogenically-induced oral wounds

Oral mucosal wounds, such as post-extraction sockets, surgical mucosal wounds, or ulcers, generally heal faster than skin wounds with less scar formation (Szpaderska et al., 2003; Vezeau, 2000). This, however, does not mean that their healing is always complication-free. Impacted healing and infection may result in pain and more frequent

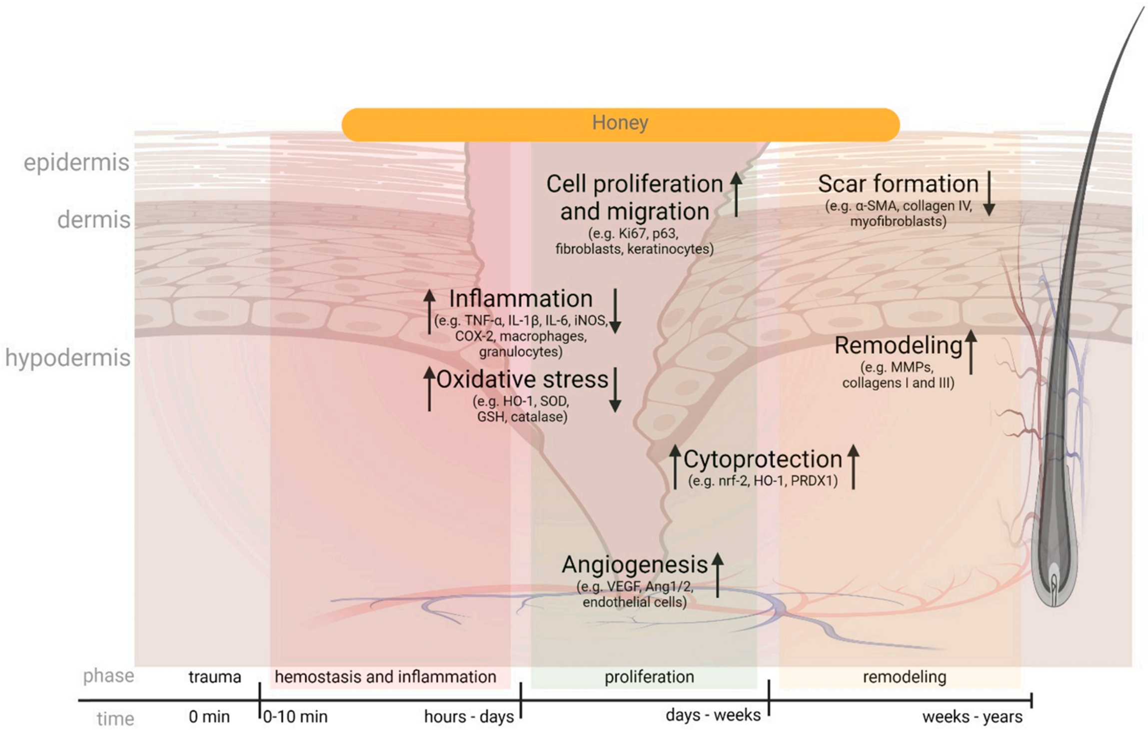

Fig. 1. Concept of how topical application of honey in wound care can influence the main processes during the hemostasis/inflammation, cell proliferation, and remodeling phases of wound healing, that partially overlap. Cellular and molecular processes can be differentially modulated by honey, dependent on the phase. Created with biorender.com.

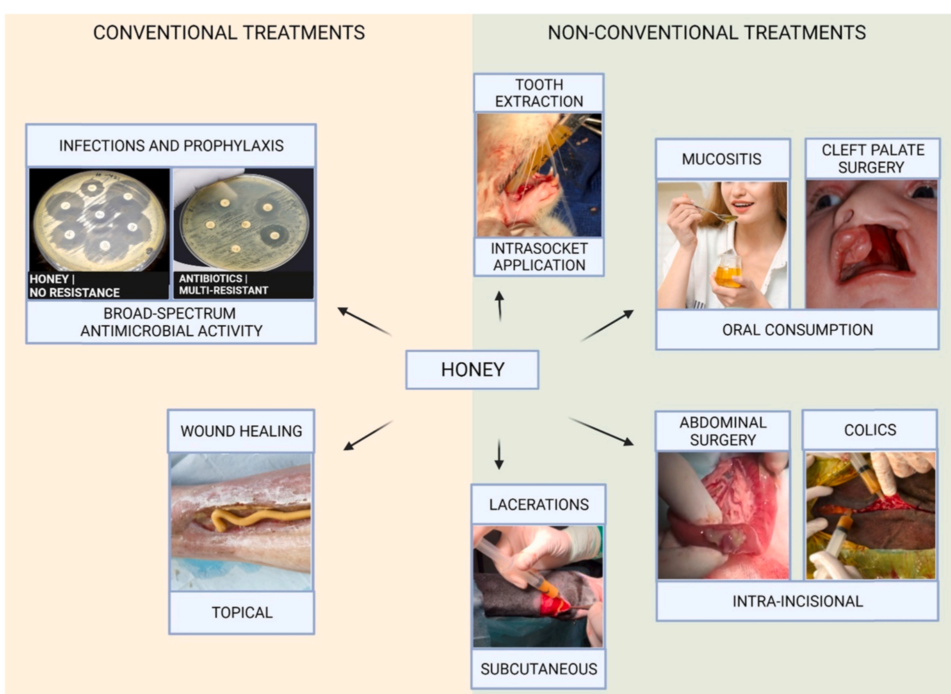

Fig. 2. Overview of conventional and non-conventional treatments with honey and examples illustrating the different routes of administration. Created with biorender.com.

visits to the dentist or doctor, leading to increased use of painkillers and antimicrobials and a larger socio-economic burden on the health care system (Alexander, 2000; Szpaderska et al., 2003; Vezeau, 2000).

Two comparable experimental studies on the use of honey to improve post-extraction socket healing could be retrieved from the literature (Table 1), one performed in rats and one in rabbits (Ilyas et al., 2015; Sarraf et al., 2019). In the rat study, 24 animals received extraction of the left first maxillary molar, after which the study group was treated with local Ziziphus honey intra-socketly. In the control group, the socket was left to fill with coagulum. The sockets of both groups were

Table 1
Studies of iatrogenically-induced oral wounds treated with honey. * and + means that the studies are overlapping.

Study design	Species	Total study size	Application	Application frequency and therapy duration	Outcome	Type/origin of honey
Tooth extraction sockets						
Experimental (Sharje, 2015)	Rat	24	Submucosal	Single	Fewer blood vessels, more bone trabeculae	Ziziphus honey
Experimental split-mouth design (Sarraf et al., 2019)	Rabbit	6	Submucosal	Single	More fibroblasts, more bone trabeculae	Nepalese honey
RCT (Ayub et al., 2013)	Human	100	Topical intra-socketly	3 times a day, 14 days	Less infection, no faster healing	Local Pakistani honey
RCT (Mokhtari et al., 2019)	Human	51	Topical intra-socketly	Single	Faster reduction in wound size	Iranian-Kurdistan Mountain honey
Prospective single arm (Soni et al., 2016)	Human	54	Topical intra-socketly	Dressing change on day: 1, 2, 3, 5, 7	Quick healing of the socket with evidence of granulation tissue	Local botanical honey
RCT (Abu-Mostafa et al., 2019)	Human	100	Topical mouthwash	Singly day 1, day 3	No significance between honey and chlorhexidine intra-socketly	Manuka honey
RCT (Al-Khanati and Al-Moudallal, 2019)	Human	66	Submucosal	Single	Less pain, faster healing	Manuka honey
Tonsillectomy						
Meta-analysis (Hwang et al., 2016)	Human	264 (4 studies)*	Topical	Variable	Less pain and improved healing	Multiple origin
RCT (Nanda et al., 2016)	Human	40*	Topical	5 mL, 6 times a day 14 days	Less pain, less NSAID intake, less fever	Local Indian honey
Prospective RCT (Ozlugedik et al., 2006)	Human	60	Topical	Daily every hour 14 days	Less pain, faster epithelization of tonsillar fossa	Flower honey, (gum tragacanth and thyme honey)
Palatoplastic donor site						
Prospective cohort (Kreshanti et al., 2012)	Human	48	Topical on the denuded maxillary bone	1 mL, 5 times a day, until epithelization of the denuded maxillary bone	Faster epithelization in the honey group	Nusantara®, local honey
Follow up (Kreshanti et al., 2018)	Human	20	–	–	More maxillary growth, suggesting less scar tissue formation	
Mucositis						
Review (Munstedt et al., 2019)	Human	17 studies+	Topical/oral	Variable	Aid in the prevention of mucositis	Multiple origin
Meta-analysis (Yang et al., 2019)	Human	1265 (17 studies)+	Topical/oral	Variable	Effective and safe in reducing mucositis prevalence and severity	Multiple origin
Meta-analysis (Liu et al., 2019)	Human	1267 (19 studies)+	Topical/oral	1–3 times/ day during radio-chemotherapy	Less severe mucositis, less pain, faster healing	Multiple origin

The * means that the RCT is cited in the review. The + marks studies with overlapping references.

surgically closed afterward. Half of the animals from each group were euthanized on day 7 post-extraction; the remaining half of the animals from each group were euthanized on day 21 post-extraction (Ilyas et al., 2015). For the rabbit study, six animals received bilateral extraction of the first mandibular premolar with unilateral intra-socket treatment with Nepalese honey followed by surgical closure of both sides. All animals were euthanized on day 7 post-extraction (Sarraf et al., 2019). After euthanasia, both studies evaluated the healing of the treated and untreated sockets histologically. The post-extraction socket filled with honey showed more bone formation in rats and rabbits than the control sockets ($P < 0{\cdot}01$) (Ilyas et al., 2015; Sarraf et al., 2019). After 21 days, the rats' extraction sockets were already filled more with bone tissue than the untreated sockets ($P = 0{\cdot}0001$) (Ilyas et al., 2015).

Several clinical trials in humans studied the effect of honey on post-extraction sockets (Table 1), but they all differ in treatment protocol and follow-up intervals, thus hampering comparison. In the first published randomized controlled trial (RCT), 20–40 years old patients were included if they needed non-surgical extraction of the first or second molar. After extraction, the socket was left open for second intention healing. Patients in the treatment group were told to apply honey (local table honey, provided by the researcher) in the open socket two-three times a day for 14 days. At 10 days post-extraction, fewer inflammatory signs such as redness, edema, and halitosis were found ($P < 0{\cdot}05$), but wound size was not significantly different between the honey-treated and control group. On day 21, there were no longer significant differences between the treatment groups (Ayub et al., 2013). A more recent RCT (Mokhtari et al., 2019), in 4–9-year-old children needing extraction of one deciduous molar tooth, quicker healing of the post-extraction socket was found when treated with honey. After extraction, wound margins of the open socket were measured as a baseline, before the single application of local Iranian-Kurdistan Mountain honey. On days 3 and 7, wound margins were measured again and compared to baseline. On both days, relative wound sizes were significantly lower after honey application than in the control group ($P < 0{\cdot}05$) (Mokhtari et al., 2019). A prospective single-arm study applied gauzes soaked in botanical honey for seven days to cure the post-extraction complication of dry-sockets in adult patients. It showed quick healing of the socket with diminished pain (measured by pain scale indexes) and evidence of granulation tissue with minimal swelling and erythema in seven days, observed by the clinician (Soni et al., 2016). The study also mentioned a swift drop in systemic CRP blood levels, suggesting less systemic inflammation. However, the lack of a control group makes the results difficult to interpret. In a randomized clinical parallel trial, intra-socket application of Manuka honey on day 1 and day 3 was compared to a twice-daily chlorhexidine 0.2% mouthwash, evaluating the healing of open sockets in adult patients undergoing extraction of a single molar tooth. The study did not find any significant benefit in healing, pain, or infection on days 3 and 7 post-extraction after honey treatment compared to mouthwash (Abu-Mostafa et al., 2019). On the contrary, the results instead suggested, although insignificantly, the inferiority of Manuka honey compared to chlorhexidine. However, the much lower application frequency of honey versus chlorhexidine was disregarded. Also, administration of NSAIDs was part of the treatment plan in all participants, potentially masking the beneficial anti-inflammatory effects attributed to honey, that are not effective in chlorhexidine (Al-Mamary et al., 2002; Pyrzynska and Biesaga, 2009). A randomized split-mouth design study included patients needing bilateral extraction of the third molar. One side of the mouth was treated intra-socketly with Manuka honey whilst the control side did not receive honey (Al-Khanati and Al-Moudallal, 2019). The treated side was chosen at random and further randomization determined which side of the mouth was extracted first. Extractions were performed 14 days apart to exclude interfering effects. On the treatment side, the honey was applied intra-socketly before the closure of the socket by suturing the mucoperiosteal flap. The mucoperiosteal flap was sutured over the extraction socket without honey treatment on the control side. Patients had lower pain scores ($P < 0{\cdot}05$) one and two days post-surgery when treated with honey. Also, the total NSAIDs intake was lower in the first 7 days after surgery ($P = 0{\cdot}0001$) (Al-Khanati and Al-Moudallal, 2019).

Honey can also help in lowering postoperative pain and improve healing after tonsillectomy in children, as a meta-analysis, which included four RCTs (Table 1) (Hwang et al., 2016). The patients had lower pain indexes in the first day post-surgery and needed fewer painkillers in the five days post-surgery, compared to the placebo group. Also, less fever was observed in the post-surgical period (Nanda et al., 2016). Furthermore, significantly faster healing and epithelization of the tonsillar fossa were observed in the 20 days after surgery ($P < 0{\cdot}001$) (Ozlugedik et al., 2006).

Cleft palate is a congenital malformation in which speaking problems and abnormal maxillary growth frequently remain because of post-operative scar formation despite surgical management such as flap palatoplasty (Simamora et al., 2012; Spauwen et al., 1993). A prospective human cohort study, in which oral honey drops were used to heal the denuded cortical bone from the donor site after flap palatoplasty showed a 2.1-fofaster epithelialization than the control group who had no treatment ($P < 0{\cdot}001$) (Kreshanti et al., 2012). In the follow-up study, the honey-treated group seemed to have better maxillary growth than the untreated group, suggesting less scar formation (Kreshanti et al., 2018). However, both the small sample size (10 treatment and 10 control) and the incomplete maxillary growth at the time of measurement should be taken into account.

For cancer, the combination of radio- and chemotherapy is a common treatment modality in the head and neck region with a possible inherent side effect of otorhinolaryngeal mucositis. This means ulceration of the oral, nasal, and/or esophageal mucosa due to mucosal atrophy and breakdown. This side effect causes pain and discomfort leading to less food and water intake and subsequently weight loss, malnutrition, and/or dehydration if not well-managed. These adverse effects can prompt dosage reduction or even discontinuation of the radio-chemotherapy in some cases. One systematic review and two recent meta-analyses, which included 17 and 17 and 19 RCTs, respectively, are published on the use of honey in patients undergoing radio- and chemotherapy (Table 1) (Liu et al., 2019; Munstedt et al., 2019; Yang et al., 2019). It has to be noticed that these studies used several overlapping references. Both meta-analyses concluded a more rapid healing of patients suffering radio- and chemotherapy-induced mucositis, even in severe cases. Interestingly, honey also showed prophylactic properties, as oral mucositis occurred less frequently in the head and neck region when patients received honey from the start of their radio- and chemotherapy. Patients reported less oral pain whilst food intake was better in the study groups. This led to increased patients' willingness to complete radio-chemotherapy. The included RCTs used honey of different origins. Remarkably, the beneficial effects of honey were less convincing in four RCTs where honey of Manuka origin was used. These studies concluded that this type of honey did not aid in preventing and managing radio- and therapy-induced mucositis (Munstedt et al., 2019). Munstedt et al. explained this by the fact that the antimicrobial component of Manuka honey, MGO, can be cytotoxic at higher concentrations, resulting in slower wound healing (Munstedt et al., 2019). To the best of our knowledge, this is the first domain where this difference in efficacy between different types of honey is evident. However, no RCT has yet compared 'non-peroxide' honey directly with 'peroxide' honey.

The oral use of honey logically raises concerns about pro-cariogenic activity due to its low pH and high sugar content. However, none of the presented studies reported this as an adverse event and honey did not affect dental erosion (Mokhtari et al., 2019; Singhal et al., 2018). By contrast, anti-cariogenic effects are reported and honey can even decrease plaque formation because of its antibacterial activity against common cariogenic bacteria, such as *Streptococcus mutans*, *Lactobacilli* and *Porphyromonas gingivalis* (Ahmadi-Motamayel et al., 2013; Atwa et al., 2014; English et al., 2004).

Summarizing (Table 1), honey has numerous positive effects on all types of wounds in the oral cavity, from surgical wounds to ulcerative wounds. Not only do wounds seem to heal faster, but also pain and patient discomfort are lower using honey. The application route and the frequency are far from standardized in literature, and the ideal treatment regimen still needs to be discovered.

Prevention of intra-abdominal adhesion formation after serosal trauma

Surgery of the abdominal cavity of humans, both by laparotomy or laparoscopy, leads to a high risk of intra-abdominal adhesions; the incidence of human peritoneal adhesions can be as high as 93% (Alpay et al., 2008; Menzies and Ellis, 1990). In the 10 years after laparotomy, 34% of the patients were readmitted due to complications related to abdominal adhesions (Ellis et al., 1999). Therefore, many anti-adhesive agents have been intensely studied to find a solution to this matter (Tingstedt et al., 2007). From the literature, four experimental animal studies could be extracted (Table 2) in which honey showed protective abilities against intra-abdominal adhesion formation. Since rats and dogs are less susceptible to post-surgical abdominal adhesions than humans due to anatomical and physiological differences, the experimental studies in those species used proven adhesion models to induce adhesions systematically and repetitively (Wiseman, 2000). Two comparable experimental studies were conducted in rats and dogs where the application of pure local honey on damaged serosal surfaces significantly reduced post-surgical abdominal adhesions ($P < 0{\cdot}05$) (Aysan et al., 2002; Shokouhi et al., 2006). In the rat study, trauma was induced under general anesthesia by rubbing a sterile gauze on the serosal surface of the cecum and 10 cm of small intestine proximal to the cecum until subserosal petechiae formed. Next, the arteries of these segments were clamped for 1 min to induce ischemia (Aysan et al., 2002). After this procedure, 5 mL of Turkish pine tree honey was applied directly on the damaged serosal surface after which the abdomen was closed. The control group received no treatment. The animals were euthanized 10 days after this procedure, and intra-abdominal adhesions were scored macroscopically by Evans's scoring system (Evans et al., 1993), ranging from no adhesions to adhesions that can only be separated by dissection. After 10 days, 30% of the honey group had no adhesions, whereas all animals had adhesion formation in the control group. For the rats in the honey group which developed adhesion formation, the adhesions were less densely formed and contained less fibrotic tissue than in the control group ($P < 0{\cdot}001$) (Aysan et al., 2002). In the dog study, a 3×4 cm area of the descending colon was rubbed with a sterile gauze and 10 mL of local unpasteurized Iranian honey was applied. The animals were euthanized after 21 days and the adhesions were also macroscopically scored by Evans's scoring system. Also, in this study, the honey group had significantly less or less severe adhesion formation ($P < 0{\cdot}05$) (Shokouhi et al., 2006). Another study in rats used not pure but diluted local Iranian honey with saline on the damaged serosal surface of the

Table 2
Effect of honey on intra-abdominal adhesion formation after serosal trauma.

Study design	Species	Size (n)	Application	Application frequency and therapy duration	Outcome	Type/origin of honey
Experimental (Aysan et al., 2002)	Rat	40, 2 groups	Intra-abdominal	Single	Fewer or less severe abdominal adhesions	Turkish pine tree honey
Experimental (Shokouhi, 2006)	Dog	18 2 groups	Intra-abdominal	Single	Fewer or less severe abdominal adhesions	Local Iranian honey
Experimental (Rahimi et al., 2017)	Rat	30 5 groups	Intra-abdominal	Single	Fewer or less severe abdominal adhesions and lower systemic inflammation levels	Local Iranian honey
Experimental (Celepli et al., 2011)	Rat	40 4 groups	Oral	Daily 21 days	Less and lower severity of abdominal adhesion formation. Lower systemic inflammation levels and higher antioxidant levels	Turkish table honey

cecum (Rahimi et al., 2017). Thirty rats were divided into five groups: negative control without any surgical procedure (normal group), a control group treated with normal saline, an experimental group treated with 1 mL of 10% honey, an experimental group treated with 5% honey, and a positive control group receiving 1 mL of dextrose 5%. After 7 days, adhesions were scored macroscopically and graded similarly to the previously mentioned studies. Both honey groups had less or less severe adhesions than the other groups ($P < 0{\cdot}001$) (Rahimi et al., 2017). Moreover, other parameters, such as inflammatory cytokines, angiogenic factors, and antioxidant levels, were measured in the peritoneal fluid seven days after the procedure. A significant ($P < 0{\cdot}001$) decrease in both the honey 5% and 10% group was found compared to control for: TNF-α, IL-1β, IL-6, TGF-β1, VEGF, NO, MDA (malondialdehyde), and an increase in GSH. Since the 5% dextrose group had a similar outcome as the negative control group for both adhesion scores and blood parameters, the sugar content in honey is thus likely not responsible for the anti-adhesive effects (Rahimi et al., 2017). Fascinatingly, oral consumption of honey demonstrated similar protective effects on abdominal adhesions as when applied on the serosal surface (Celepli et al., 2011). This study in rats induced adhesion formation by rubbing a sterile gauze on the cecum followed by dissection of a peritoneal patch of 1 × 1 cm of the abdominal wall opposite the abraded cecal area (Celepli et al., 2011). One group of rats received Turkish table honey (4 g/kg/day), one group received bee pollen (4 g/kg/day), and one group honey and bee pollen (both 2 g/kg/day). The treatment was given daily by a gastric tube for 21 days post-surgery. The control group received no additional treatment or oral supplementation. The rats were euthanized and the adhesions were scored macroscopically. The control group performed significantly worse than the honey, the pollen, and the honey and pollen group (P-value 0.025, 0.035, and 0.025, respectively). Dense adhesions were not seen in any of the treatment groups. Further, this study looked at various oxidative stress and inflammatory parameters in liver tissue and blood on day 21. When rats were fed honey or honey and pollen, MDA (oxidative stress parameter) was lower whereas; glutathione peroxidase, superoxide dismutase (SOD), and catalase (anti-oxidant enzymes) were higher compared to the control group ($P < 0{\cdot}05$ for all). The reduction of oxidative stress and inflammation in the pollen group was less convincing, although that might have been dose-dependent (Celepli et al., 2011). The researchers also suggested that the anti-adhesive effect of honey could be mediated by increased anti-oxidative and anti-inflammatory effects downstream of ingestion of honey.

The rat studies mentioned above showed that honey has anti-adhesive effects in the abdominal cavity after direct application and after ingestion. Nevertheless, the tested volume of honey directly applied on the serosal surface was far less than the tested daily ingested dose. Extrapolating a dose of 4 g/kg/day/rat to an average human would implies that the patient needs to consume a small jar of honey daily. However, it would be interesting to further investigate whether consuming a lower dose of honey daily would be advantageous."

Subcutaneous administration of honey improves wound repair

Apart from the oral and intra-abdominal application of honey to facilitate surgical wound repair, there have recently been two provocative studies performed in horses (Table 3) in which honey was applied subcutaneously (Gustafsson et al., 2020; Mandel et al., 2020). A large prospective, open-label randomized block design clinical study studied the intralesional application of honey (L-Mesitran soft®) in horses with skin lacerations followed by primary wound closure (Mandel et al., 2020). The honey-treated wounds were more likely to heal completely ($P = 0{\cdot}006$) and were less likely to have signs of inflammation like redness or edema ($P = 0{\cdot}007$). The authors postulated that the antibacterial effects of subcutaneous honey clear the affected tissues from bacterial contamination and subsequently aid the wound repair from deeper within the wound (Mandel et al., 2020). Another study in horses was recently published on SSI of the abdominal incisional wound after abdominal surgery treating colic disease (Durward-Akhurst et al., 2013), notoriously known for their high incidence of wound infection and dehiscence in horses (Durward-Akhurst et al., 2013; Gustafsson et al., 2020; Mair and Smith, 2005). In that RCT, honey (L-Mesitran soft®) was applied in the wound bed after the closure of the *linea alba* with a quantity of 0.5 mL/cm incision length. Then, closure of the subcutis and cutis was performed (Gustafsson et al., 2020). With that straightforward subcutaneous application, the rate of post-surgical wound infection went down from 32·5% in the control group to only 8·1% in the honey-treated group, underlining the enormous prophylactic benefit of honey. The mortality rate of 28·3% (15/53) in the control group was strongly decreased in the treatment group to 10·9% (6/55), with a relative risk of 0.3855 ($p = 0{\cdot}0314$). This seems to be a significant decrease in mortality in the honey-treated group (Gustafsson et al., 2020). As the mortality rate was not discussed in the paper, we unfortunately do not know the causes of the mortality. We therefore cannot identify the role of honey in this matter.

Both equine studies show that a single subcutaneous application of honey during the closure of wounds has potent protective effects on later wound infection/inflammation in horses. Subcutaneous administration would therefore be attractive for translation into human medicine. Both studies note that the honey was easy to apply to the surgical site without apparent effects on the suture material.

Biochemical and molecular mechanisms explaining the healing-promoting mechanisms of honey

In this review, the use of honey in non-conventional indications shows the broad applicability and potency outside of its regular scope as an antimicrobial agent and as a topical wound therapeutic (Fig. 2). The topical application of honey in wound care is widely accepted, but other non-conventional modes of administration may offer novel potent solutions for different indications. The outcomes of these studies are mainly supported by clinical evidence, but basic research can enhance our understanding of the underlying molecular, biochemical, and cellular mechanisms.

Table 3
Studies with subcutaneous application of MGH.

Study design	Species	Size (n)	Application	Application frequency	Outcome	Type of honey
Prospective open-label randomized block design clinical trial (Mandel et al., 2020)	Horse	126, Tx 69 C: 57	Subcutaneous	Single	Wounds are more likely to heal with fewer signs of infection and dehiscence	L-Mesitran soft®
Prospective randomized clinical trial (Gustafsson et al., 2020)	Horse	89, Tx: 49 C: 40	Subcutaneous	Single	Less wound infection and dehiscence	L-Mesitran soft®

Tx: Treatment group, C: control group

Honey has beneficial molecular properties on wound repair that directly support the different processes illustrated in Fig. 1 during the different wound healing phases. Honey can stimulate a pro-inflammatory response by activating TLR4 signaling, which is necessary during the inflammatory phase to attract leukocytes, including granulocytes and macrophages, subsequently eliminating the debris in the wound bed and protecting against pathogens (Rodriguez et al., 2008). As explained above, honey also protects directly against a wide variety of pathogens, even in biofilms, and it has prophylactic activity. Honey drives immunomodulatory actions on the wound because of its cytokine releasing effect on cells within the wound area, such as monocytes/macrophages, neutrophils, fibroblasts, endothelial cells, and keratinocytes. In the inflammatory phase of wound healing, the production of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) enhances the first inflammatory reaction, critical to wound repair (Majtan, 2014a, 2014b; Tonks et al., 2007). Honey seems to first enhance this pro-inflammatory response before it subsequently suppresses the production of these pro-inflammatory cytokines by downregulating NF-κβ and MAPK pathways and promoting the resolution of inflammation (Koh and DiPietro, 2011; Ranneh et al., 2021; Raynaud et al., 2013; Sindrilaru and Scharffetter-Kochanek, 2013). Honey also lowers the formation of reactive oxygen species (ROS) apart from reducing wound inflammation (Majtan, 2014a, 2014b; van den Berg et al., 2008). Honey activates, as a feedback mechanism, NRF2-target genes, including heme oxygenase (HO-1), peroxiredoxin (PRDX1), SOD, glutathione reductase, and catalase mediating an anti-inflammatory and anti-oxidant response (Alvarez-Suarez et al., 2016; Dong et al., 2008; Kassim et al., 2010; Kassim et al., 2012; Majtan, 2014a, 2014b; Ranneh et al., 2019; Sun et al., 2020; Tonks et al., 2007; van den Berg et al., 2008). During the cell proliferation phase, honey stimulates the proliferation of new dermal cells, such as keratinocytes, fibroblasts, and endothelial cells, and the production of extracellular matrix (ECM) proteins to promote tissue regeneration (Fig. 1) (Guo and Dipietro, 2010). In the clinical setting, this can be observed by a decrease in the five cardinal signs of inflammation (i.e. rubor, tumor, calor, dolor, function loss).

Interestingly, honey can thus both drive and attenuate inflammation. This is likely dependent on the phase of wound healing, the microenvironment, and the composition of honey components. Honey consists of multiple different components, including carbohydrates, flavonoids, amino acids, vitamins, and minerals (Hermanns et al., 2020). Numerous compounds contribute to the marked antioxidant and anti-inflammatory activity, such as flavonoids, phenolic acids, tocopherols, ascorbic acid, and enzymes, including catalase (CAT) or superoxide dismutase (SOD) and Maillard reaction like products, such as MGO (Al-Mamary et al., 2002; Brudzynski and Miotto, 2011; Pyrzynska and Biesaga, 2009).

Components of honey can act as a danger signal and activate the immune system via TLR4 signaling. Exposure of macrophages to honey promotes pro-inflammatory cytokines release, unrelated to possible LPS contamination (Raynaud et al., 2013). A 5.8 kDa molecule in honey was shown to be responsible for activation of TLR4, but not TLR2, in human monocytes, resulting in TNF-α production. Blocking TLR4 but not TLR2 significantly reduced honey-stimulated TNF-α production by human monocytes. As proof of principle, honey-stimulated cytokine production was evaluated in macrophages from wild type, TLR2, and TLR4 knockout (KO) mice. Honey-stimulated TNF-α production was observed in wild type and TLR2 KO macrophages but not in TLR4 KO cells (Tonks et al., 2007). Keratinocytes showed increased mRNA levels of the pro-inflammatory cytokines TNF-α, IL-1β, TGF-β, and MMP-9 in the cytoplasm when treated with honey, which was followed by collagen IV matrix degradation, a step linked to the migration of keratinocytes over denuded epithelial surfaces (Majtan et al., 2010). (Fig. 1).

Antioxidant systems reduce the adverse effects of ROS and reactive nitrogen species (RNS), inhibit the NADPH oxidases responsible for producing superoxide anions, act as metal chelators, and interfere with the chain reactions of free radicals (Scepankova et al., 2021). Flavonoids from honey, such as chrysin, apigenin, quercetin, inhibit pro-inflammatory enzymes like cyclooxygenases (COX), lipoxygenase, cytochrome P450, and consequently, the formation of pro-inflammatory cytokines. Additionally, flavonoids in honey also reduce pro-inflammatory gene expression by inhibiting the activation of NF-κβ and p38-MAPK in the cytosol (Abdel-Latif and Abouzied, 2016; Kim et al., 2018; Ranneh et al., 2019; Silva et al., 2021).

An important process during the proliferation phase is cell proliferation, migration, and wound contraction as these will help fill the gap in the wound. *In vitro* wound healing studies on fibroblasts and keratinocytes supported that honey enhances wound closure by cell proliferation (Ki67, p63) and migration (scratch assay) (Barui et al., 2013; Martinotti et al., 2019; Ranzato et al., 2012, 2013). The source of honey may influence the healing potential, as Manuka honey increased fibroblast migration but was significantly lower than buckwheat honey and acacia honey in wound scratch assays. This could be explained by differential activation of signaling pathways in the fibroblasts and keratinocytes (Ranzato et al., 2012, 2013). Acacia honey mainly activated the ERK pathway, buckwheat honey activated both the ERK and p38 MAP kinase pathways, whereas Manuka honey mainly activated the p38 MAP kinase pathway. The least activated pathway was PI3K for all tested honey sources (Ranzato et al., 2013). Also, a difference in interleukin expression was seen, where acacia and buckwheat, but not Manuka honey, induced significant increases in the release of interleukin-4 (IL-4), IL-6, and IL-8 (Ranzato et al., 2013). This suggests that one can tune up and optimize the efficacy of the treatment by adjusting the honey composition to the different needs of the patient.

A proper vasculature structure is important for supplying oxygen and nutrients, and fostering rapid tissue repair (Rademakers et al., 2019). *In vitro* studies with endothelial cells showed that honey dose-dependently stimulated tubule formation and wound healing (scratch assay) (Ranzato et al., 2021; Rossiter et al., 2010). Consistently, honey has pro-angiogenic activity in a murine diabetic wound model and can induce the expression of the prime angiogenic factors VEGF and VEGFR-II (Chaudhary et al., 2020). In addition, honey was shown to promote sequential stages of wound healing, including wound closure, re-epithelialization, and collagen I and III deposition (Chaudhary et al., 2020).

During the proliferative phase, α-smooth muscle actin-positive myofibroblasts excrete ECM molecules, such as collagens, fibronectin, proteoglycans, and elastin, which are necessary for normal wound healing. However, a prolonged persistence of myofibroblasts, due to the presence of inflammatory and oxidative stress, leads to excessive scar formation due to the continued production of ECM molecules and contraction (Bochaton-Piallat et al., 2016). Honey may facilitate apoptosis of myofibroblasts (Chaudhary et al., 2020). By targeting the redox balance, honey can create a pro-healing microenvironment that optimizes scarless wound healing (Wagener et al., 2013). Also, exposure of keratinocytes to honey increases the production of matrix metalloproteinases (MMPs), such as MMP-9, and influences the degradation of collagen IV, which is important during the remodeling phase (Majtan et al., 2010).

Finally, honey and some of its flavonoids have anti-nociceptive and analgesic effects, which could experimentally be reversed by mu-receptor antagonists, suggesting that they act like opioid analgesic drugs (Zakaria et al., 2015).

Potentials and limitations of honey in iatrogenically caused wound care

Historically, honey is much researched and praised for its antiseptic properties. The topical use of honey in skin wound care is widely known (Morris and Cerceo, 2020). Here, we searched for studies that describe the use of honey in wound care outside the already well-known topical dermal application. All the studies mentioned in Tables 1–3 show that honey has various advantages in managing these wounds, such as potent antimicrobial activities, attenuated wound inflammation, faster wound healing, and less dehiscence while relieving pain discomfort. No adverse, harmful, or side effects were mentioned in the cited studies. However, short-term itchy or stingy sensation is reported in the literature related to applying pure honey on open wound beds (Oluwatosin et al., 2000). Warning, raw, unprocessed table honey could have adverse effects because of the possibility of contamination with herbicides, pesticides, heavy metals, antibiotics, and bacterial spores.

On the contrary, medical-grade honey (MGH) is processed and tested to prove its safety and efficacy for its use in medical wound care (Hermanns et al., 2020). The MGH is also gamma irradiated to inactivate bacterial spores of which *Clostridium botulinum* is most prevalent in raw honey (Hermanns et al., 2020; Mohd Tamrin, 2020). Gamma-irradiation is preferred over heat sterilization as the latter process also inactivates the enzymes in the honey (Cooper and Jenkins, 2009; Hermanns et al., 2020). Honey consists of over 200 components, including enzymes, amino acids, carbohydrates, vitamins, organic acids, mineral compounds, and other derivatives from the environment where the nectar was collected (Hermanns et al., 2020). This inevitably leads to variable consistency and activity. With this in mind, honey approved to be of medical-grade quality should preferentially be used in research studies for its safety and insight should be given into the chemical composition to make studies more repeatable and comparable.

Both included horse studies show that a single subcutaneous application of MGH strongly decreased the infection rate and facilitated wound healing (Gustafsson et al., 2020; Mandel et al., 2020). Due to its antiseptic properties, MGH quickly clears wound surfaces of bacterial colonization, thereby making SSI less likely to occur. The advantage of the subcutaneous application is that the MGH can be applied under sterile surgical conditions. Next, the wound can be surgically closed, while MGH can protect the deeper tissues against pathogens. It is critical to understand more about the diffusion of honey from its application site into wound tissue. This could help understand whether the subcutaneous application has beneficial effects on wound healing in deeper tissues than topical application. No studies have been published yet indicating how long honey remains at its application site or how deep honey diffuses into the deeper wound tissue. Studies on this matter could advance understanding of wound management and the application of MGH to wounds.

Different honey types exhibit differential immunomodulating effects on the dermal fibroblasts (Ranzato et al., 2013). The clinical difference between peroxide-based honey and non-peroxide-based honey is only visible in the mucositis meta-analysis, where non-peroxide-based honey results were less profound than the peroxide-based honey. Even honey derived from the same plant origin could have different anti-inflammatory potential. Two different samples of Bracatinga honeydew honey were both inhibitory to the production of NOx, IL-6, TNF-α, MCP-1, IL-12p70, IFN-γ, and IL-10, however, to differential extents explained by the diversity of the phenolic compounds between both samples (Silva et al., 2020). This underlines the large variety of different kinds of honey and their possible effects on study outcomes. Finally, supplements added to honey could enhance antimicrobial activities as two recent studies show that co-exposure to vitamin C and E had a synergistic impact against *Pseudomonas* biofilms.[66,67] This adds up to the already existing variety in honey types. When these individual effects of the honey composition are better characterized, this could offer the opportunity to finetune a specific response (Combarros-Fuertes et al., 2020). Selecting between different types of honey depending on the indication and needs, e.g., a stronger anti-inflammatory, antimicrobial activity or adding supplements may also help provide personalized medicine.

Traditionally, MGH was primarily known for its antiseptic properties; however, the studies mentioned above show a wider range of additional benefits besides the antiseptic properties. The RCTs demonstrate the effects of MGH on inflammation, bone growth, healing, pain perception, and anti-adhesive effects on the serosal surface. In none of the cited studies, adverse side effects or allergic reactions were reported.

Conclusion

Honey has antimicrobial activities targeting multidrug resistant pathogens and enhancing wound healing. This review provided compelling evidence that honey can also revolutionize the treatment of non-conventional indications as demonstrated by the activation of critical signaling pathways. Apart from the traditional topical application, alternative routes of administration can prevent infections and promote wound repair. To ensure the most optimal selection of honey regarding its safety and efficacy, standardized and quality-checked MGH is recommended. However, the current literature still lacks studies conducted with MGH and individual study protocols show variation. Further research is encouraged to expand the promising clinical applications of MGH outside the regular scope and more emphasis on research on the driving molecular mechanisms of honey components. We expect that MGH can be as groundbreaking as Semmelweis' handwashing and prevent infections of conventional and non-conventional applications in the future.

Declaration of interests

NAJC is employed by Triticum Exploitatie BV. Triticum Exploitatie BV is the manufacturer of the MGH-based formulation L-Mesitran. This review focuses on honey in general and not on the range of L-Mesitran products. We ensure to have maintained the highest standards of integrity and presented the work in an unbiased manner. Other authors state no conflict of interest.

Significance Statement

Honey exerts strong antimicrobial and wound healing activities, making it a potent new treatment option for non-conventional indications, i.e. other than topical skin wounds.

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