MANAGEMENT OF BIOFILMS IN ACUTE AND CHRONIC ULCERS BY LOCAL APPLICATION OF HONEY.

By

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Dissertation submitted to

BLDE UNIVERSITY VIJAYAPUR

In partial fulfilment of the requirements for the degree of

MS

In

GENERAL SURGERY

Under the guidance of

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DR ANAND SAGAR RAGATE.

LIST OF ABBREVIATIONS USED

SL.NO	-	SERIAL NUMBER
N	-	NAME
A	-	AGE
S	-	SEX
M	-	MALE
F	-	FEMALE
IP.NO	-	IN PATIENT NUMBER
DOA	-	DATE OF ADMISSION
DOS	-	DATE OF SURGERY
DOD	-	DATE OF DISCHARGE
STSG	-	SPLIT THICKNESS SKIN GRAFTING
EC	-	ESCHERICHIA COLI
EF	-	ENTEROCOCCUS FECALIS
KO	-	KLEBSIELLA OXYTOCA
KP	-	KLEBSIELLA PNEUMONIAE
SA	-	STAPHYLOCOCCUS AUREUS
LFNHU	-	LEFT FOOT NONHEALING ULCER
LLNHU	-	LEFT LEG NONHEALING ULCER
RHNHU	-	RIGHT HAND NONHEALING ULCER
RFNHU	-	RIGHT FOOT NONHEALING ULCER
RLNHU	-	RIGHT LEG NONHEALING ULCE

ABSTRACT

With the introduction of new antimicrobials into clinical practice, the emergence of resistant strains of bacteria normally follows at some point. Resistant species tend to dominate in environments where antimicrobial agents are in common use. Antimicrobial resistance not only threatens to increase the cost of health care and jeopardise health care gains to society, but it may even impact the economy (WHO, 2012).

A wound on the body of a person is a major concern to the patient. Wounds that heal in less than 30 days are known as acute ulcers. Wounds or ulcers which don't heal within 30-60 days are loosely termed as chronic wounds or ulcers. One of the most important reason for an ulcer to become chronic is due to formation of biofilm[1.]

Honey on the wound bed not only draws material out of the wound, but also prevents biofilm formation and cross-contamination. It provides a barrier effect on an open wound preventing further infection from external contamination.

METHOD OF COLLECTION OF DATA:

All patients admitted at Shri B M Patil Medical college Hospital and Research Centre , Vijayapur with symptoms/ clinical features of acute and chronic ulcer during the period of October 2013 to June 2015 were taken up for study.

- Patients with symptoms and / clinical features of acute and chronic ulcers were taken up for the study.

- History of patients will be noted and detailed examination of the ulcer was done. Ulcers were evaluated on presentation /surgery and labelled acute or chronic.

RESULTS:

In our study of Management of Biofilms in acute and chronic ulcers with local application of honey, we found that out of total 60 patients mean age was of 54.5 ± 16.6 in years. In 60 ulcers with biofilm, staphylococcus aureus was present predominantly in 58.3% patients. The mean duration for eradication of biofilm in this study was 18.1 ± 5.0 days. All patients were discharged with a mean duration time of 26.4 ± 3.1 days.

CONCLUSION:

All patients admitted for acute and chronic ulcers with biofilm were effectively managed by local application of honey with significant reduction in the hospital stay.

KEY WORDS: Honey, Biofilm, Granulation tissue, Split thickness skin graft, Eradication time.

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INTRODUCTION

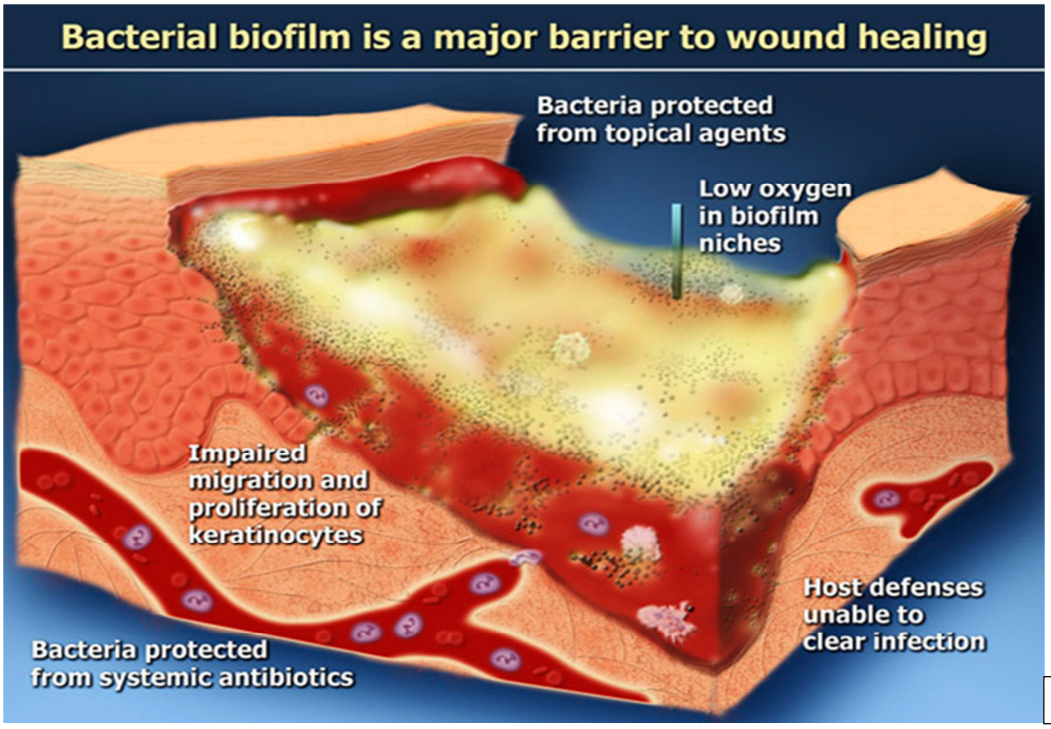
With the introduction of new antimicrobials into clinical practice, the emergence of resistant strains of bacteria normally follows at some point. Resistant species tend to dominate in environments where antimicrobial agents are in common use. Antimicrobial resistance not only threatens to increase the cost of health care and jeopardise health care gains to society, but it may even impact the economy (WHO, 2012).

A wound on the body of a person is a major concern to the patient. Wounds that heal in less than 30 days are known as acute ulcers. Wounds or ulcers which don't heal within 30-60 days are loosely termed as chronic wounds or ulcers. The wound healing is delayed for many reasons like lack of adequate blood supply, adequate sensation, adequate nutrition, adequate rest, anaemia and biofilm formation. Apart from the common reasons, one of the most important reason for an ulcer to become chronic is due to formation of biofilm[1.]

Bioburden on the wound bed may be one of the most important barriers to wound healing. The bioburden comprises devitalized tissue, proteinaceous exudate, white blood cells and, most specifically, microorganisms. Given that surface-associated bacteria organize into biofilm, it appears that they are the most important component of the wound bioburden. [2]

Research on wound bacteria has traditionally focused on planktonic cells. However, biofilms may be totally different from the 'planktonic' or free-floating bacteria that we have come to understand. Indeed, our misunderstanding of the physiology, genetics, physical and biochemical properties of bacteria found within

wound biofilms may result in misguided management such as sequential treatments, low-dose short-term antibiotics and antiseptics, and prolonged treatment with a single biocide. [3]



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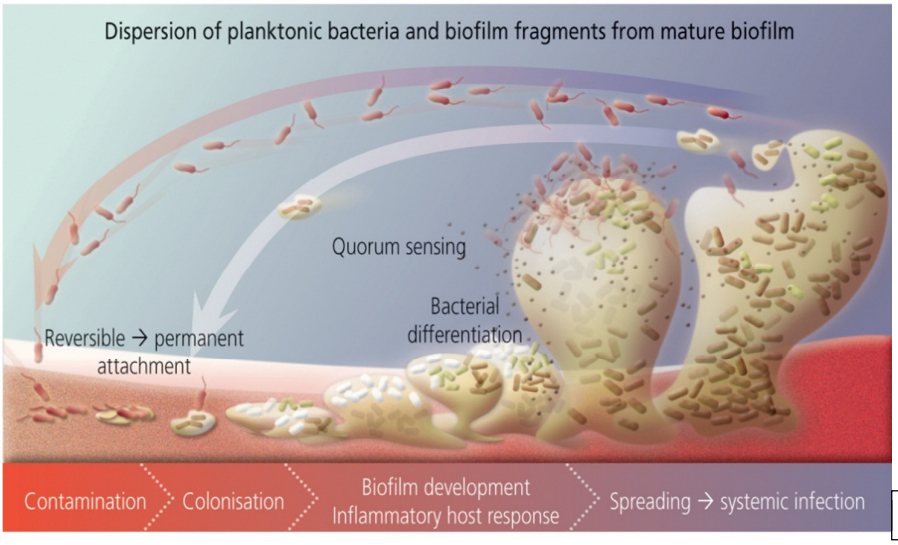
Bacteria isolated from chronic wounds are generally cultivated and studied using traditional methods that relate to bacteria in the planktonic state.[4] Once isolated they are concentrated in pure cultures, cultivated in nutrient-rich media, identified and their antibiotic-resistance profiles established.[5,6] However, planktonic bacteria grown in the laboratory are thought to behave differently to bacteria located on the wound surface. This is because microorganisms in the chronic wound bed are considered to exist predominantly within a biofilm community.[7] A common medical paradigm for bacteria on the wound surface is termed the 'contamination-infection continuum'.[8] This suggests that individual bacteria land on the wound surface (contamination), find

nutrient sources and begin to multiply, replicating outside the host and utilizing nutrients on the wound surface (colonization).

Once the individual bacteria have multiplied to reach a critical mass (critical colonization), they can become recalcitrant to standard clinical therapies[1]. As microorganisms, principally bacteria, within a wound continue to replicate, they begin to invade the host. If the bacteria are able to invade host tissue and are highly virulent, the tissue becomes infected. This model projects what we know about the behavior of single-cell microbes (planktonic) into our view of the wound bioburden. Naturally occurring bacteria attached to surfaces rarely behave like planktonic bacteria.[9] The contamination-infection continuum model, which reflects the planktonic paradigm, needs to be updated to take account of biofilms.[10]



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The National Institutes of Health (NIH) has estimated that up to 80% of human infectious diseases are biofilm related[8]. More than 99% of bacteria found in nature exist in these stable, persistent biofilms, and there are reasons to believe this bacterial theme also holds true in the wound environment [2,4,8]. Bacteria encountered in nature and medical diseases are commonly located on a surface, but function in multi-species communities held together by an extracellular slime, known as extracellular polymeric substances (EPS). This slime is composed of polysaccharides, proteins and nucleic acids, and often makes up 80% of the biofilm. The remaining 20% are microbial cells that reside within a microbial community encased within the EPS matrix[11,12].

The members of the biofilm community possess different genotypic and phenotypic traits, resulting in a structure that is heterogenous, dynamic and recalcitrant to antimicrobials and the immune response.[13] Antibiotics fail to eradicate biofilms due to poor penetration, metabolic inhibition, protected quiescent bacteria (persisters) and other mechanisms. *In vitro* investigations have shown that bacteria in mixed-species biofilm communities can act synergistically in ways not observed in planktonic bacteria.[14-16] This will, no doubt, change the way clinicians view infection. Chronic biofilm infections, such as catheter infections, endocarditis and osteomyelitis, often persist indefinitely unless the infected material is removed.[17-20] This persistence is also evident in chronic wounds. For example, venous leg ulcers can remain open for years, possibly because the host response is unable to clear the biofilm infection. In such cases, it is plausible to suggest the biofilm obtains nutrients not from devitalized tissue, but from plasma and other exudate percolating from the wound bed. The biofilm may even associate closely with blood vessels and so modulate the host's inflammatory response.[21-24] An inadequate blood supply to the infected area – as in a

diabetic foot ulcer, results in decreased host response, increased biofilm virulence and tissue necrosis.[25] It is possible that the biofilm can manipulate the level of the inflammatory response by modulating its chemical appearance and altering its cell-to-cell signaling activity.[26-29]

Cell-to-cell signaling activity takes place through a quorum-sensing pathway. Quorum-sensing molecules are continuously secreted from each individual bacterium, and act on the same bacterial species, interspecies and even on the cells of their mammalian host. For *Pseudomonas aeruginosa*, acyl-homoserine lactone (AHL) is one of the first discovered and best-known quorum-sensing molecules. When a critical density of bacteria is present, sufficient quorum-sensing molecules accumulate to upregulate dedicated biofilm pathways and express biofilm phenotype virulence factors, dramatically changing the phenotype of the bacterium.[30] The quorum-sensing pathway can express over 800 new proteins not seen with planktonic phenotype bacteria.[30]

Quorum-sensing inhibitors such as brominated furanones, which occur naturally in the red algae Delisia, can block the receptors for AHL and its isotypes furanones. This holds great promise for the eventual management of medical biofilms. Incorporating biofilms into the model for microbial infection and wound chronicity may better explain the biochemistry and cellular biology of the chronic wound environment.[31] For example, chronically elevated pro-inflammatory cytokines (tumour necrosis factor-alpha, interleukin-1, alpha and gamma interferons), increased matrix metalloproteases levels (MMP-2, 8 and 9) and increased elastase can be explained by the possible effects of a biofilm on the host's innate immune system.[32]

Biofilms may also influence fibroblast senescence, keratinocyte impairment and the failure of endothelial cells to initiate angiogenesis.[33]

All open wounds, because they lack the protective covering of skin, contain microorganisms from endogenous (the patient's own flora) or exogenous sources. In the early stages of the formation of a chronic wound, these microbes are generally held in check or destroyed by the host's immune system. However, if microbes get attach to the wound surface and proliferate, a biofilm will begin to develop. When the biofilm is well established, it will exhibit resistance to destruction by the host immune system and antimicrobials. At this stage, the biofilm is considered mature and more difficult to eradicate. When this occurs, the wound is defined as being in a biofilm infected state.[34]

To understand any biofilm infection, it is necessary to understand its life cycle. A biofilm is initiated when a planktonic bacterium or a fragment of biofilm (cluster of diverse cells embedded in an intercellular matrix) irreversibly attaches to an appropriate surface,[35] such as the exposed extracellular matrix of a wound or an implanted medical device.[36] Once bound, the bacteria divide and form a microcolony of cells. When a critical density is reached, secreted pheromones (quorum-sensing molecules) and the altered environment within the biofilm cause phenotypic alterations in the bacterial community. The microcolony thus becomes a robust biofilm community that is recalcitrant to the host immune system and to many therapeutic interventions.[37]

Significant alterations occur during biofilm maturation. For example, during the development of a monoculture biofilm, more than 50% of the protein expressed by the bacteria can differ several-fold, depending on the biofilm's stage of

development.[38] This enhanced expression of proteins is thought to aid biofilm resistance to antimicrobials and the host's immune response. The biofilm's strengths are found in its heterogenicity (different protein expression), interspecies cooperation and intercellular matrix structure.[18] The most metabolically active cells in the biofilm are located near the non-attached surface where they grow, reproduce, slough and behave similar to planktonic cells. These metabolically active cells are the most vulnerable to the effects of antibiotics, antiseptics and host defences.

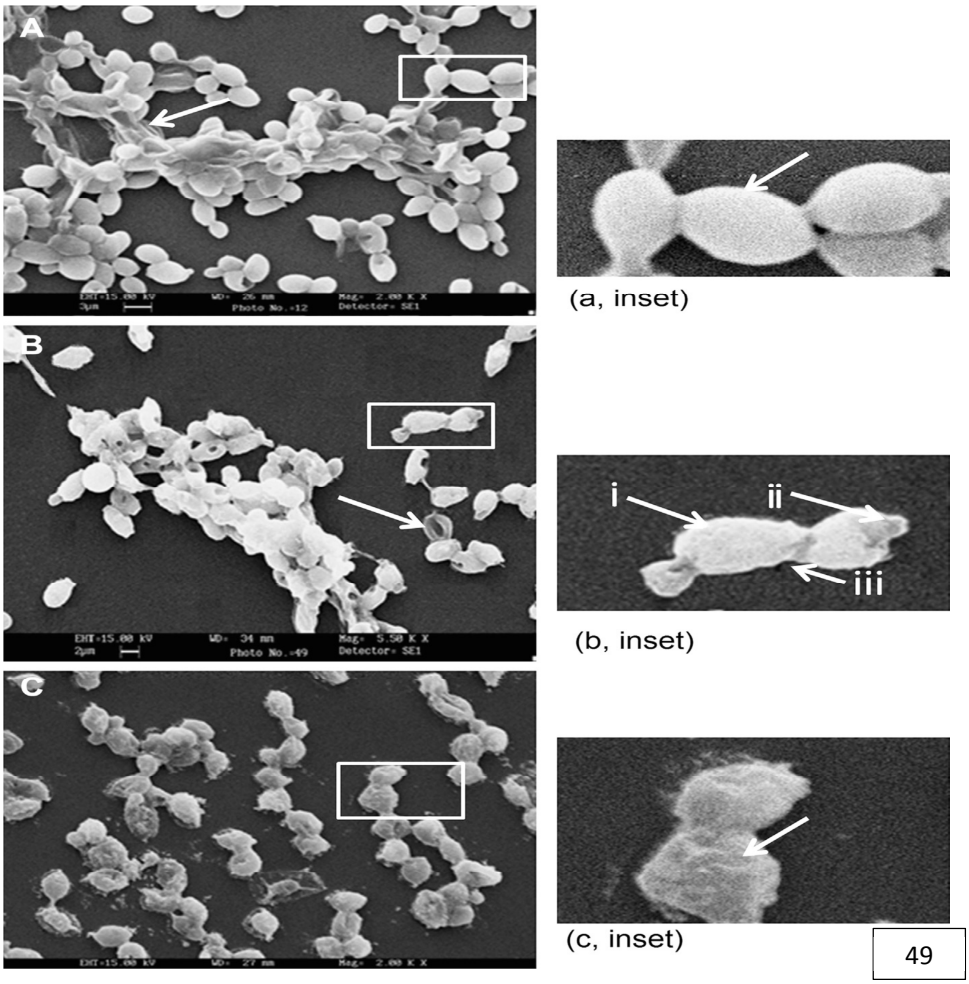
Bacteria that are more deeply embedded in the biofilm's extracellular matrix are sheltered from external perturbations, less metabolically active and more resistant to an array of antimicrobial therapies. [39-42] These protected bacteria can reconstitute the community should a stress destroy the more vulnerable cells at the biofilm surface.[8,9]

It is this ability to remain viable in spite of stresses and to adapt and reconstitute itself that makes the biofilm so tenacious. The developed biofilm harbours physical and metabolic defences that enable it to resist antimicrobials that typically annihilate planktonic cells.[43-46] These defences include resistance to:

- Ultraviolet light
- Biocides
- Antibiotics
- Host defences.[5,13,22]

Consequently, managing a biofilm community is more difficult than treating planktonic bacteria. In our opinion, multiple and synchronous strategies are therefore the most effective way of combating a biofilm infection.[47]

The incidence of biofilm in a chronic ulcer is 60% whereas in an acute ulcer it is only 6%.[1] It has been estimated that biofilms are associated with 65% of nosocomial infections and that treatment of biofilm-associated infections costs more than $1 billion annually in United States.[48]



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Scanning electron microscopy micrographs of the 48 h C. albicans biofilms on microtiter plates. (A) Biofilm formed in the absence of honey, showing a dense network of cells and hyphae. White arrow indicated exopolysaccharides material (A, inset). White arrow indicates the smooth cell wall of a normal cell. (B) Inhibition of established biofilm treated with 40% w/v of jujube honey (after 24 h) is illustrated.

There is no exopolysaccharide material observed and white arrow indicates the formation of small pores within the cell walls (B, inset). i, white arrow indicates the rough cell wall; ii, vesicle formation due to lytic material; iii, shrinkage in cell membrane due to plasmolysis of cell. (C) Prevention of biofilm formation on microtiter plates after 48 h is illustrated. (C, inset). White arrow shows rough cell wall and shrinkage in cell membrane due to plasmolysis of cell.

Honey has many purposes in medicine. Historically it has been used to treat coughs, asthma symptoms, and even blood pressure.[4] Long before the discovery of bacteria, it was considered to be the oldest wound dressing[3] as it dates back to ancient medical writings of Egypt, Greece and parts of India.[50] Its use on skin wounds has been documented on skin grafts, trauma wounds, necrotizing fasciitis, pilonidal sinuses, pressure ulcers, lacerations, burns, surgical wounds, herpetic lesions, atopic dermatitis, animal bites, and rheumatoid ulcers.[51] The use of honey was forgotten with the discovery of antibiotics. However, with antibiotic resistance on the rise in recent years, honey has been rediscovered and its uses are once again being investigated.

Honey is a natural, sweet substance produced by honey bees of the genera Apis and Meliponinae. The bees collect nectar from a variety of flowers and process it by adding their own body enzymes and deposit it into wax cells of the hive where it is concentrated by evaporation through fanning of the bees' wings. The final result is a supersaturated sweetener composed of 80% sugar and 17% water. The remainder of the honey is made up of proteins, enzymes, and non-essential amino acids.[52] The high sugar concentration is primarily composed of simple sugars which include 38.2 % Fructose and 31.3 % Glucose that are readily absorbed by the body and the other sugars such as Maltose 7.35%, Sucrose 1.3% and Isomaltose make up the additional 30%. [5]

The enzymes found in honey, play an important role in its antibiotic properties. Invertase produced by the bee converts sucrose to glucose and fructose, amylase breaks down starch, glucose oxidase converts glucose to gluconolactone which in turn yields gluconic acid and hydrogen peroxide.[53] Trace amounts of vitamin B, calcium, iron, zinc, potassium, phosphorus, magnesium, selenium, and chromium are also found in the composition of honey. The low pH of honey comes from the organic acids acetic, butanoic, formic, citric, succinic, lactic malic, pyroglutamic, and gluconic acid.[54]

Although the exact composition of honey varies depending on the geographical source and the plants on which the bees have been feeding, this supersaturated mixture of sugars with small quantities of enzymes, amino acids, vitamins, minerals and organic acids holds many desired properties for an impressive antibacterial dressing for wounds.[55] Several studies have shown it to inhibit over 60 species of bacteria including anaerobes, gram-positive and gram-negative bacteria, and even some yeast species of *Aspergillus*, and *Penicillium*.[56]

The super-saturated solution of honey, containing only 17% water inhibits bacterial growth primarily due to this high osmolarity. Water is essential for the survival of bacteria but with a low availability the microorganisms cannot survive and reproduce. When honey is applied to wounds, the high solute concentration creates an osmotic effect drawing lymph and other fluid out of the wound bed diluting the honey.[58] As the osmolarity decreases by the wound drainage, the antibiotic activity is not lost and at times is increased as Sackett11 noted in his study. It is the enzymatic effect from glucose oxidase's production of hydrogen peroxide that brings about the continuous additional antimicrobial effect after the sugar saturation is lost. Glucose

oxidase secreted from the hypopharyngeal glands of the bee, converts glucose to gluconic acid and hydrogen peroxide. The bactericidal effect of hydrogen peroxide, further decreases the number of microorganisms available on the wound bed. The release of hydrogen peroxide is slow and continuous for a constant antibacterial effect successfully eliminates microorganisms but is not cytotoxic to the surrounding tissue.[59]

Another factor associated with the antibiotic effect of honey is thought to be due to the phytochemicals in the nectar. The phytochemicals found in honey mostly consist of complex phenol and organic acids that further serve an antibacterial function.[20] They also aid in reducing the risk of oxidative damage in the tissue. The concentration of phytochemicals varies depending on the plant source of the nectar and makes some honey's more effective than others in terms of their antimicrobial activity.[17-21]

The third antimicrobial property of honey is due to glucose oxidase converting glucose to gluconic acid which gives honey its low pH.[10,11] Honey has an acidic composition with a pH between 3.2-4.5, acidic enough to inhibit many pathogens.[10,11,17] More the acidic pH, more is the pathogen growth inhibited. In addition to decreasing the pathogens in the wound, the acidic environment is beneficial to epithelialization. The acid environment increases the amount of oxygen released from the hemoglobin in the wound bed, which, in turn, increase the rate of granulation.

Honey in microscopy has shown to reduce inflammatory cells in acute and chronic inflammation. Although its exact mechanism is not understood, it stimulates peripheral blood to draw B and T lymphocytes to the surface and activates phagocytes

even at honey concentrations as low as 0.1%.[60] It also stimulates monocytes to release cytokines, Tumor necrosis factor-1, and IL 1 and 6.[60] Reducing inflammation is very important in wound healing as it improves circulation and delivers more oxygen and nutrients to help the tissue repair and heal.[7] The anti-inflammatory effects of honey also reduce the hypertrophic scarring during the maturation phase of wound healing resulting in less scar tissue.[19]

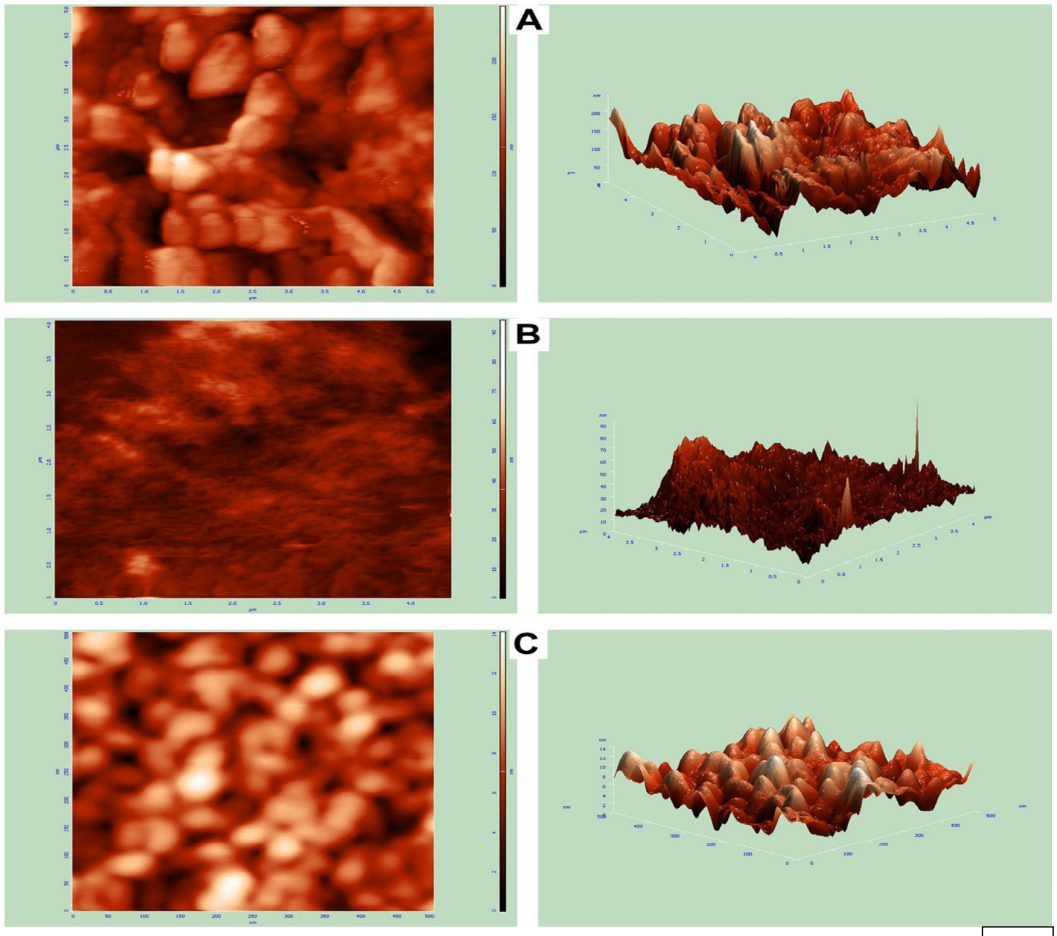
It is hypothesized that the presence of a biofilm on a chronic wound surface is a barrier to healing. When the skin is broken and a wound forms, the primary host defence to bacterial adhesion and colonization is compromised. The host defences try to prevent bacteria that seed the wound developing into a chronic infection. However, various host impairments may result in a chronic wound:[61]

- Poor perfusion
- Malnutrition
- Presence of a foreign body
- Pressure
- Repetitive trauma
- Hyperglycemia
- White blood cell dysfunction.

If a biofilm develops in a wound, its presence in the wound may be difficult to suppress, especially in an individual with a compromised immune system. [22,30] The bacteria and their extracellular components may thus be able to prolong inflammation indefinitely, delaying the normal healing process. It is the presence of a biofilm on the wound surface that, most likely constitutes its chronic state.[62]

The high osmolar concentration of honey not only works as an antimicrobial property but also as aids in debridement of the wound. The strong osmotic action draws exudates and lymph fluid from the wound towards the surface to add the moisture needed for autolytic debridement.[45]This osmotic autolytic debridement, action washes the wound base from beneath as it removes debris and sloughs off necrotic tissue that would normally slow down healing process.[3,8,10] Honey on the wound bed not only draws material out of the wound, but also prevents biofilm formation and cross-contamination. It provides a barrier effect on an open wound preventing further infection from external contamination.[63]

Honey has a unique feature which reduces and eliminates the malodor by producing antibacterial action against odor producing anaerobes such as b*acterioides spp*, *prevotella ssp, peptosterptococcus ssp*. destroys the bacteria that typically produces malodor.[5] Secondly, the glucose provided by honey metabolized by the bacteria, as an alternative of using amino acids from the metabolism of serum and dead cells. The end result is production of lactic acid, instead of malodorous ammonia, amines, and sulfur compounds which give a wound the unpleasant foul smell.[34,39]



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Atomic force microscopy micrographs showing the variation in the roughness and height of C. albicans biofilms on microtiter plates: (A) untreated biofilm after 48 h (height 200 nm). (B) 40% w/v jujube honey-treated established biofilm (48 h) (height 90 nm). (C) Formation of biofilm after treatment with 40% w/v of jujube honey (48 h) (height 14 nm).

Honey is a natural product that has been widely used for its therapeutic effects. It has been reported to contain about 200 substances. Application of honey as wound dressing leads to stimulation of healing process and rapidly clears the infection. Honey has cleansing action on wounds, stimulates tissue regeneration and reduces

inflammation. Honey impregnated pads act as non-adhesive tissue dressing. The enzyme glucose oxidase of honey provides glucose to leucocytes, which is essential for respiratory burst to produce hydrogen peroxide leading to antibacterial activity of macrophages. The acidity of honey further aids in antibacterial activity.[64]

Honey also contains Nitric oxide metabolites, which is important for healing, bacterial killing, viral inhibition, immunological response, and respiratory, renal, cardiovascular, and nervous systems functions. Many studies have implicated NO in the inflammatory and proliferative phases of wound healing[65]. Wound healing involves platelet inflammatory cell, fibroblast, and epithelial cells; all of them are capable of producing NO[68]. NO can reverse impaired healing in diabetic patients[66].

Prostaglandins are mediators of inflammation and pain. They are widely regarded as immunosuppressive, which can decrease many aspects of B- and T-lymphocyte functions[67]. It found that honey can lower plasma prostaglandin concentrations in normal individuals[68]. Its inhibitory effect was increased with time. The site of actions could be either at COX- 1 or COX-2, or both. Recently, it was found that artificial honey made of glucose and fructose increased prostaglandin concentrations[68]. Therefore, natural honey might contain raw materials that are capable of inhibiting prostaglandin synthesis[68]. The ability of honey to lower prostaglandin concentrations could explain many of its biological and therapeutic effects, particularly those related to inflammation, pain, immunity, and wound healing.

Honey increases antibody production during primary and secondary immune responses against thymus-dependent and thymus-independent antigens. The actual mechanism to stimulate antibody production was not identified. NO is an important

mediator of immune responses[69]. A single dose of L-arginine, a known precursor of NO, caused a significant increase in humoral response[70]. Therefore, honey might increase humoral immunity by means of its ability to enhance NO production.

A study conducted at Ege university school of nursing, Izmir , Turkey, Yapucu Gunes U, et al , to compare the effect of honey dressing vs an ethoxy-diaminoacridine plus nitrofurazone dressing in patients with pressure ulcers. After 5 weeks of treatment , patients who were treated by honey dressing had significantly better PUSH tool(Pressure Ulcer Score for Healing) scores than subjects treated with the ethoxy-diaminoacridine plus nitrofurazone dressing (6.55+/- 2.14 vs 12.62+/- 2.15, P<0..001). By week 5, PUSH tool scores showed that healing among subjects using honey dressing was approximately 4 times the rate of healing in the comparison group. The use of honey is effective and practical.[71]

AIM OF THE STUDY

- To detect biofilm in acute and chronic ulcers.
- To study the effect of honey on healing of acute and chronic ulcers with biofilm.

REVIEW OF LITERATURE

Biofilms were probably first recognised by Anthony Leeuwenhoek who noticed microbial attachment to his own tooth. Later on it was forgotten for nearly two centuries.

In 2000, several mechanisms were proposed to explain the phenomenon of resistance within biofilms, including delayed penetration of antimicrobial agents into the biofilm extracellular matrix, slowing of growth rate of organisms within the biofilm, or other physiological changes brought about by interaction of the organisms with a surface .[72]

In 2007, in a clinical study on chronic wounds, specimens were obtained from 77 subjects. Of these 50 chronic wound specimen were evaluated by microscopy for biofilm, 30 had biofilm (60%) and 8 acute wound specimen had biofilm (6%).[73]

In 2007, an invitro multispecies Lubbock chronic wound biofilm model was proposed. They noticed that multispecies biofilm were becoming increasingly recognized as the naturally occurring state in which bacteria reside. Three of the most important species associated with multispecies biofilm were Pseudomonas aeruginosa, Enterococcus faecalis and staphylococcus aureus. The study was conducted to address the need for a chronic pathogenic biofilm laboratory model that allows for cooperative growth of these 3 organisms. [74] One of the primary health issues that was recognised to be exacerbated by biofilms were chronic nonhealing wounds such as venous leg ulcers, diabetic foot ulcers and pressure ulcers.

In 2007, a study of biofilm-based wound management in subjects with critical limb ischemia was conducted to know healing rates in comparison with a previously published study, Biofilm Based Wound Control strategies significantly improved healing frequency. These findings demonstrate that effectively managing the biofilm in chronic wounds is a important component of consistently transforming "non-healable" wounds into healable wounds.[75]

In 2008, a study Diabetic foot ulcers observed excellent results in treating diabetic wounds with dressing soaked with natural honey. The disability of diabetic foot patients was minimized by decreasing the rate of leg or foot amputations and thus enhancing the quality and productivity of individual life.[76]

In 2009, " A comparative study of different methods to detect biofilms" was conducted which showed that the sensitivity and specificity of Tube adherence test were 100% sensitivity and 100% specificity of the Tube adherence test when compared to PCR (concomitant presence of the icaA and icaD or icaACD genes). The sensitivity and specificity were calculated using the presence of the ica genes as a parameter.[77]

In 2009, a trial of three honeys was conducted against 17 strains of *Pseudomonas Aeruginosa* isolated from wound patients to compare its antimicrobial effects. They tested Medihoney which works primarily using phytochrome as an antibiotic compared to mixed pasture honey which used hydrogen peroxide as its antibiotic activity and an artificial honey which uses the high osmolar concentration of sugar as an antibiotic source. The results showed no difference in the antibacterial effect of the natural honeys with a minimum inhibitory concentration of 6.8-7.5% but

a significantly higher concentration of 17-22% for artificial sugar is needed to inhibiting all 17 strains.[79] This demonstrates that there is an additional antibiotic characteristic apart from the high osmolarity concentration but no difference in effectiveness between the hydrogen peroxide over the phytochrome honey.[79]

With the rise in antibiotic resistance the US FDA gave clearance for the use of Medihoney as a wound dressing product in 2007. Medihoney contains Manuka honey from *Leptospermum scaparium* derived from New Zealand tea trees.[80] The honey is sterilized with γ radiation to remove the spores but retain its biologic properties.[81] Manuka honey has a high level of phytochemical components and has been found to be very effective in clearing wounds.[80] It is known that honey's antibacterial activities are slower than those of traditional antiseptics which decrease bacteria count in mere minutes but balancing the speed against honey's other properties is the question and issue here. The subject of this review was whether these studies show that its combination of longer lasting bactericidal activity, its autolytic debridement activity, moist environment formed by the lymph preventing the dressing from adhering to the wound, and the sugar content and acidic environment promoting epithelialization through the increased availability of oxygen and nutrients to the cells decrease the healing time and make it a better wound dressing.[81]

The antibacterial activity is related to four properties of honey. First , honey draws moisture out of the environment and thus dehydrates bacteria. The sugar content of honey is also high enough to hinder the growth of microbes, but the sugar content is not alone is not the sole reason for honey's antibacterial properties. Second, the pH of honey is between 3.2 and 4.5, and this acidity is high enough to inhibit the

growth of the most microorganisms. Hydrogen peroxide produced by the glucose oxidase is the third and probably the most important antibacterial component, although some authors believe the nonperoxide activity to be more important. Lastly, several phytochemical factors for antibacterial activity have been identified in honey.[82]

MATERIALS AND METHODS

SOURCE OF DATA :

All patients admitted at Shri B. M. Patil Medical college Hospital and Research Centre ,Vijayapur with symptoms/ clinical features of acute and chronic ulcer during the period of October 2013 to June 2015 were taken up for study and sample size is 60.

METHOD OF COLLECTION OF DATA:

- Patients with symptoms and / clinical features of acute and chronic ulcers were taken up for the study.
- History of patients will be noted and detailed examination of the ulcer was done. Ulcers were evaluated on presentation /surgery and labelled acute or chronic.

INCLUSION CRITERIA :

- Patients having acute and chronic ulcers.
- Patients having diabetic foot ulcers, venous ulcers and pressure sores.

EXCLUSION CRITERIA :

- Acute and Chronic ulcers in immune compromised patients.

Acute ulcers included post surgery (Debridement) and Chronic ulcers included non healing ulcers of more than 30 days. Swab culture from the ulcers was taken on day 1 and sent for biofilm detection. After Gram staining and smear examination, specimen was inoculated on blood agar and Mac Conkeys agar. The inoculated plate was incubated at 37 degree C for 18 to 24 hours (overnight incubation). These plates were observed for growth. If there was growth then the isolates were further subjected for biochemical identification and antibiotic susceptibility testing was done as standard protocol. They were tested for development of biofilm. If there was no growth, the plates were further incubated for 24-48 hours then the culture was labelled as sterile.

- Honey dressing was done for ulcers with biofilm .
- On day 1, Ulcer assessment was done with parameters like discharge, foul smell, granulation tissue and size. Dabur honey of 10-30 ml was taken on a sterile gauze piece and diluted with normal saline in ratio of 1:2 and was spread over ulcer bed thoroughly and the ulcer was covered using sterile pads and roller guaze. Consecutive days regular dressing with honey was done.

- On day 5, wound assessment was done regarding discharge from wound, smell, granulation tissue , and size .Culture swab was taken and Honey dressing was done .
- The same protocol was followed for consecutive days, ulcer assessment was done using same parameters and culture swab was taken and sent for biofilm detection.
- Honey dressing was continued till the ulcer was free from from biofilm.
- Ulcers free from biofilm were taken up for skin grafting.

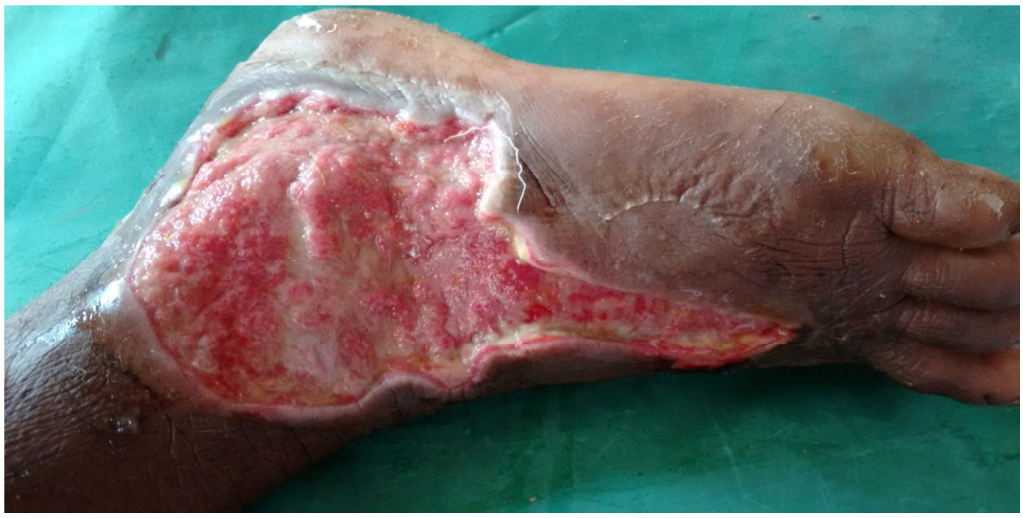


Fig 1. Wound with healthy granulation tissue on day 5 after using dabur honey.

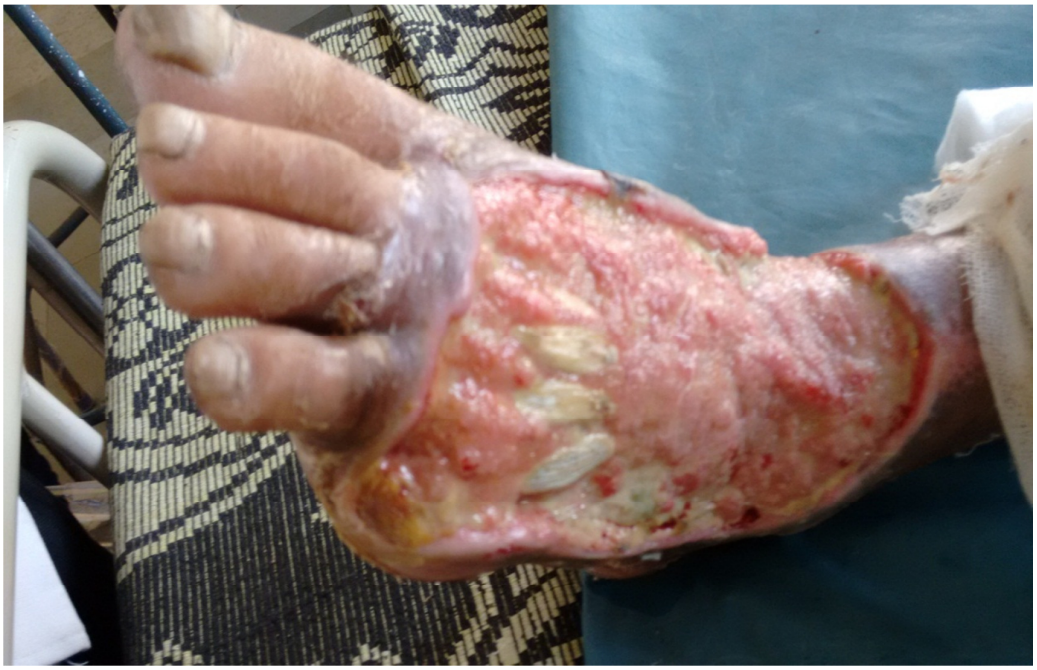


Fig 2. Day 10, Formation of granulation tissue after application of honey.

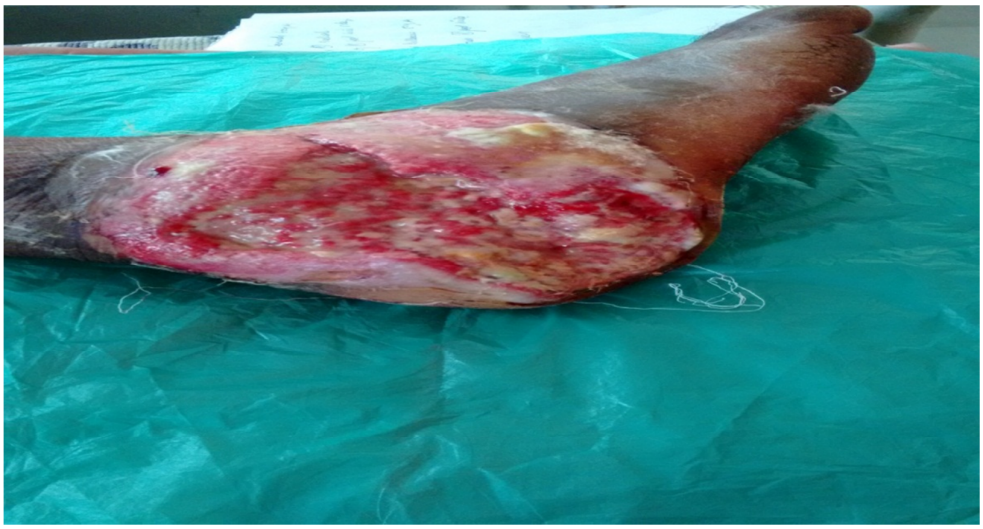


Fig 3. Day 5 Of a chronic ulcer with granulation tissue after application of honey.

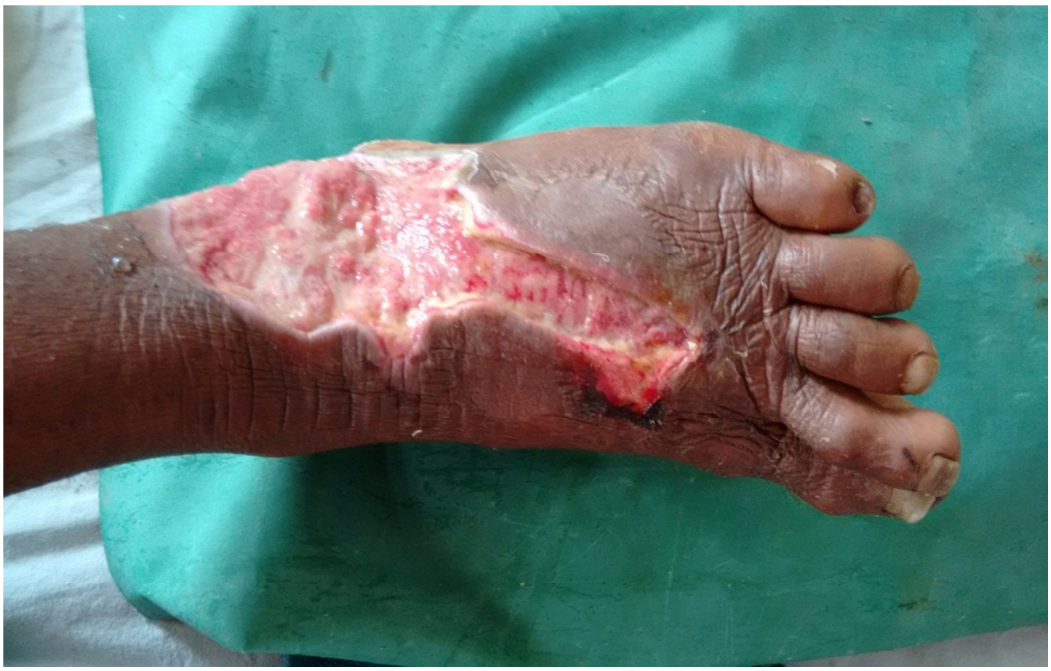


Fig 4. Day 10, chronic ulcer with granulation after application of honey.

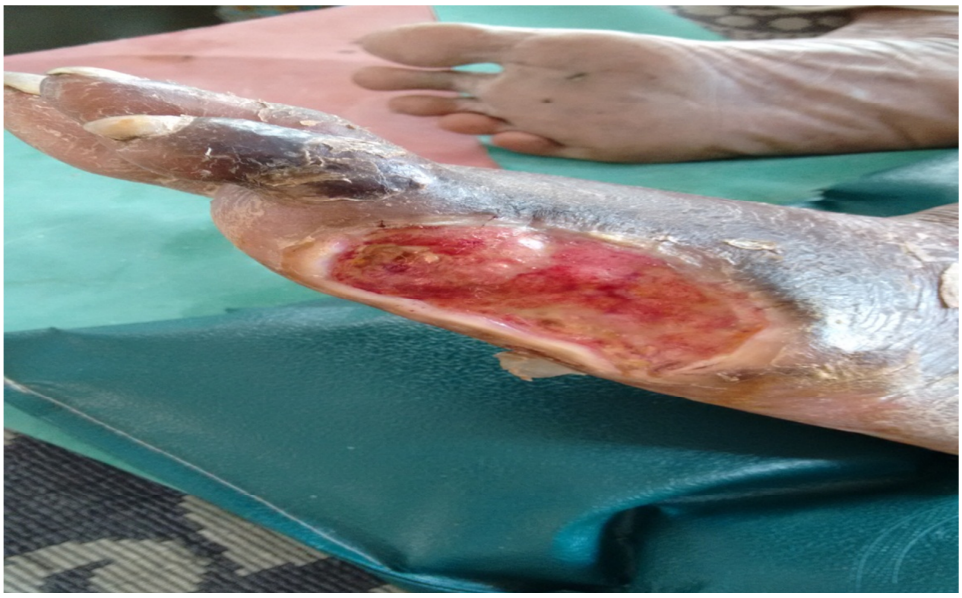


Fig 5. Day 10 with healthy granulation tissue after application of honey.

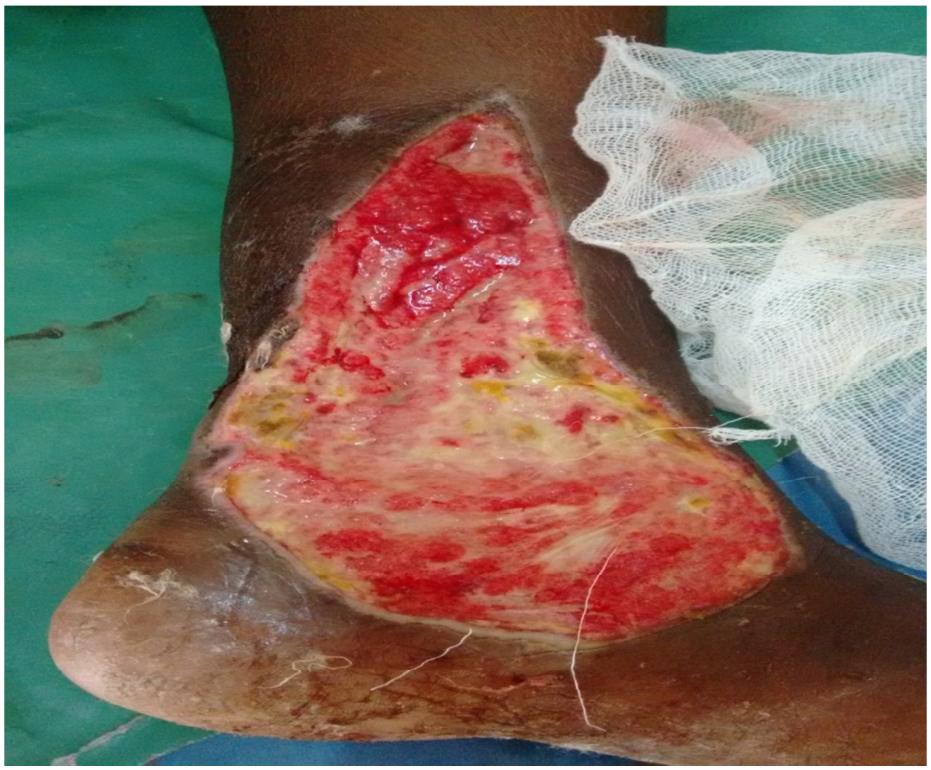


Fig 6. Day 10 with healthy islands of granulation tissue after application of honey.

SAMPLING :

Study period from: October 2013 to June 2015.

All the patients admitted during this period, who fulfil the inclusion criteria, were included in this study.

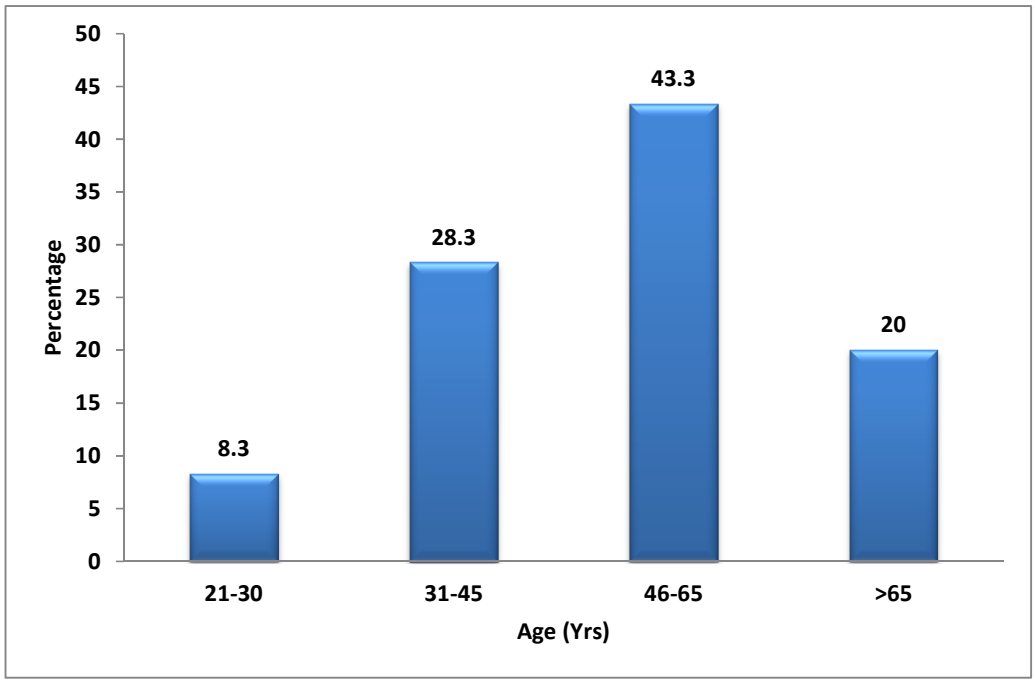
RESULTS

Table 1: Percent Distribution of Age (Yrs)

Age (Yrs)	N	Percent
21-30	5	8.3
31-45	17	28.3
46-65	26	43.3
>65	12	20
Total	60	100

In this study, the percent distribution of age was maximum from 45-65 age group.

GRAPH 1 : Percent Distribution of Age (Yrs)



Bar diagram depicting maximum patients from 45-65 age group that is 43.3 percent.

Table 2 : Mean Distribution of Age (Yrs)

	Minimum	Maximum	Mean	SD
AGE	21	88	54.5	16.6

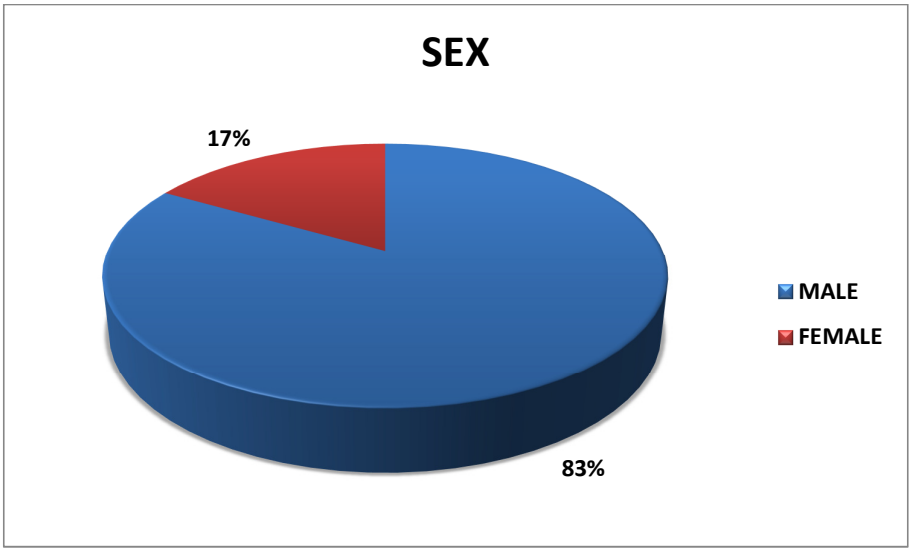
The mean distribution of age in the study was 54.5 years.

Table 3 : Percent Distribution of Sex

SEX	N	Percent
MALE	50	83.3
FEMALE	10	16.7
Total	60	100

The percent distribution of sex with male preponderance of 83.3% whereas with females 16.7%

GRAPH 2 : Percent Distribution of Sex



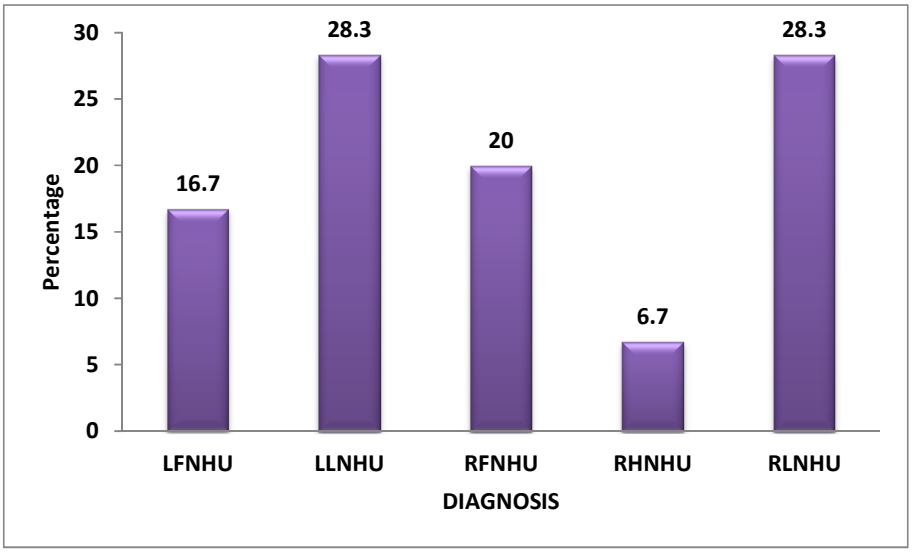
Pie diagram depicting male preponderance.

Table 4 : Percent Distribution of DIAGNOSIS

DIAGNOSIS	N	Percent
LFNHU	10	16.7
LLNHU	17	28.3
RFNHU	12	20
RHNHU	4	6.7
RLNHU	17	28.3
Total	60	100

In this study, the percentage of left leg non healing ulcers (28.3%) and right leg non healing ulcers (28.3%) was more compared to others.

GRAPH 3 : Percent Distribution of DIAGNOSIS



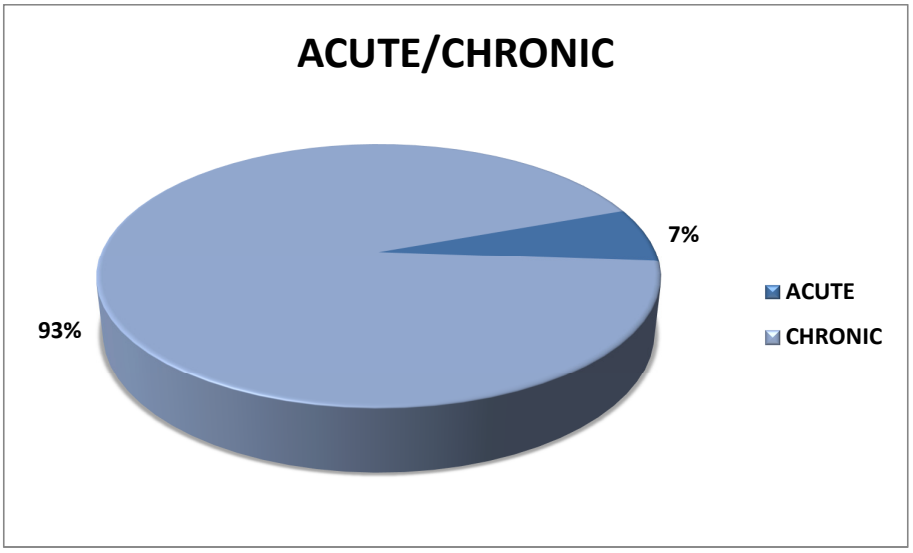
Bar diagram depicting the preponderance of LLNHU and RLNHU.

Table 5 : Percent Distribution of ACUTE/CHRONIC

ACUTE/CHRONIC	N	Percent
ACUTE	4	6.7
CHRONIC	56	93.3
Total	60	100

In the study, chronic ulcers with biofilm were 93.3% and acute ulcers with biofilm were 6.7%.

GRAPH 4 : Percent Distribution of ACUTE/CHRONIC



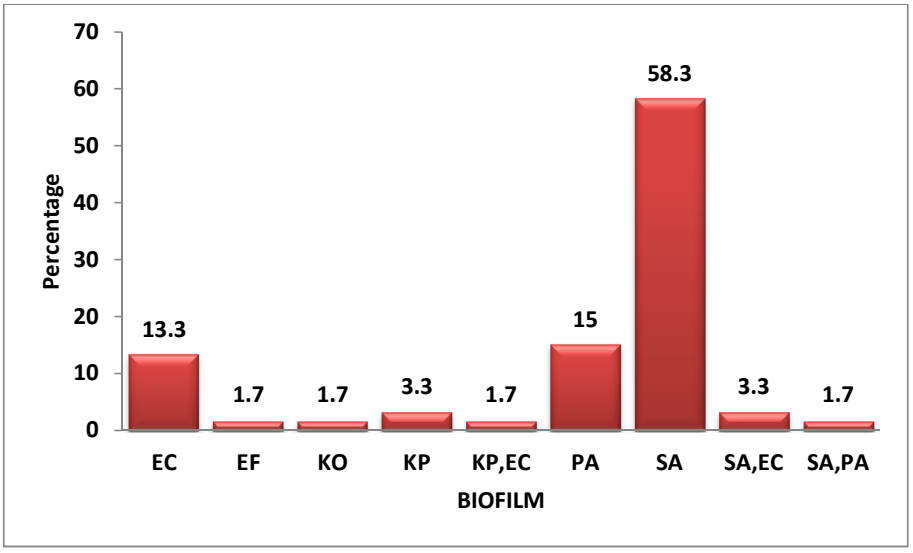
Pie diagram showing chronic ulcers of 93% and acute ulcers of 7%.

Table 6 : Percent Distribution of BIOFILM

BIOFILM	N	Percent
EC	8	13.3
EF	1	1.7
KO	1	1.7
KP	2	3.3
KP,EC	1	1.7
PA	9	15
SA	35	58.3
SA,EC	2	3.3
SA,PA	1	1.7
Total	60	100

In the study, the highest percentage was of staphylococcus aureus with 58.3% and 5% patients had multiple organisms.

GRAPH 5 : Percent Distribution of BIOFILM



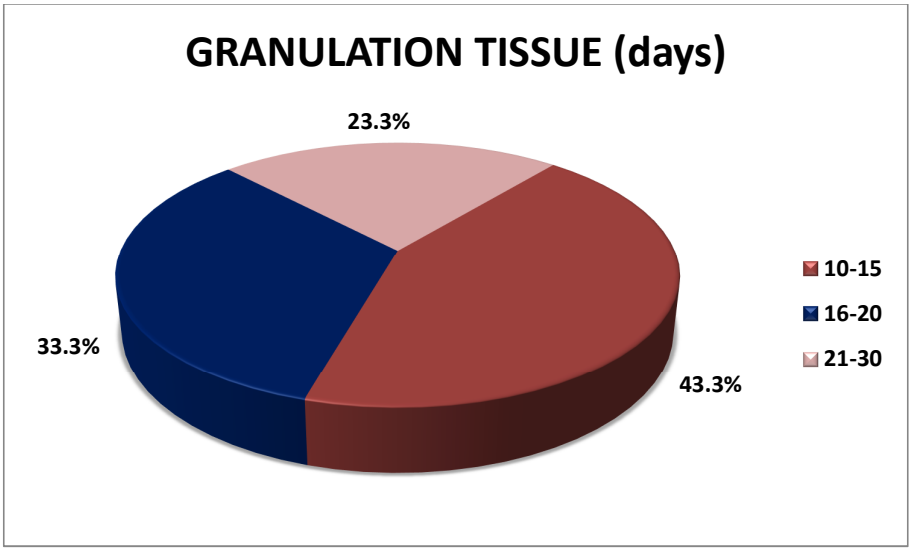
Bar diagram depicting preponderance of staphylococcus aureus with 58.3%.

Table 7 : Percent Distribution of GRANULATION TISSUE (days)

GRANULATION TISSUE (days)	N	Percent
10-15	26	43.3
16-20	20	33.3
21-30	14	23.3
Total	60	100

In the study, the formation of granulation tissue was maximum by 10-15 days with 43.3%.

GRAPH 6 : Percent Distribution of GRANULATION TISSUE (days)



Pie diagram depicting maximum patients with formation of granulation tissue by 10-15 days.

Table 8 : Mean Distribution of GRANULATION TISSUE (days)

	Minimum	Maximum	Mean	SD
GRANULATION TISSUE (days)	10	30	18.1	5.5

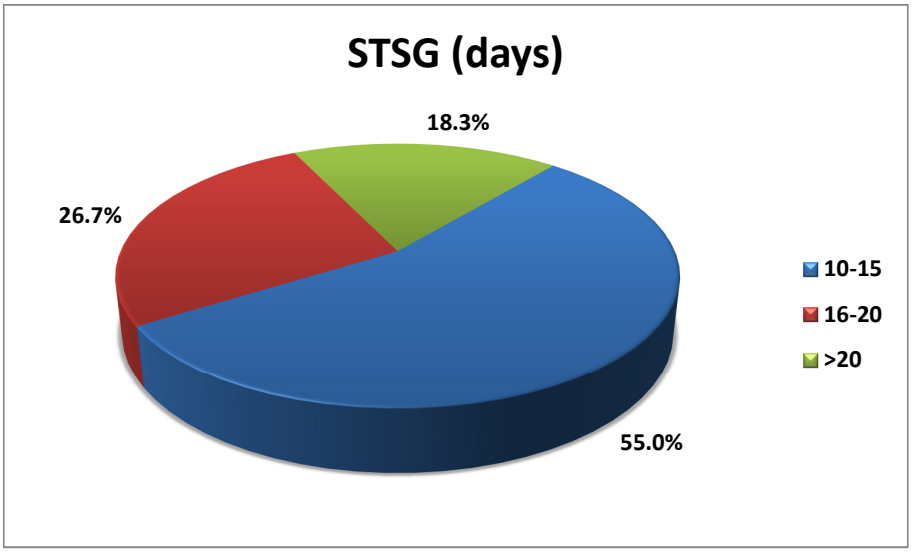
The mean duration for formation of healthy granulation tissue in this study was 18.1+/- 5.5 days.

Table 9 : Percent Distribution of STSG (days)

STSG (days)	N	Percent
10-15	33	55
16-20	16	26.7
>20	11	18.3
Total	60	100

In the study, 55% of patients underwent STSG by 10-15 days, 26.7% patients in 16-20 days, 18.3% of patients after 20 days.

GRAPH 7 : Percent Distribution of STSG (days)



Pie diagram depicting maximum number of patients (55%) underwent STSG by 10-15 days.

Table 10 : Mean Distribution of STSG (days)

	Minimum	Maximum	Mean	SD
STSG (days)	10	26	16.9	4.4

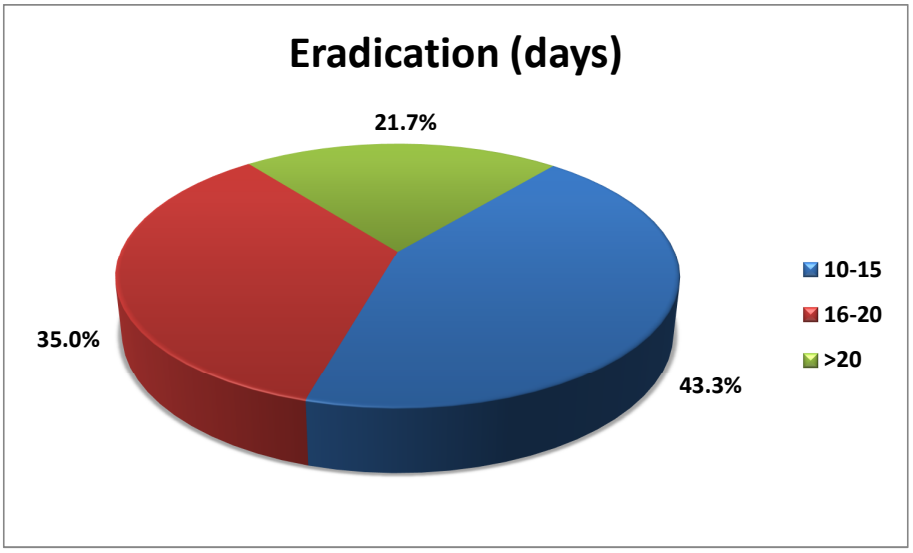
The mean duration for STSG in this study was 16.9+/-4.4 days

Table 11 : Percent Distribution of Eradication (days)

Eradication (days)	N	Percent
10-15	26	43.3
16-20	21	35
>20	13	21.7
Total	60	100

The eradication time in 43.3% of patients was 10-15 days, in 35% patients was 16-20 days and in 21.7% patients was more than 20 days.

GRAPH 8 : Percent Distribution of Eradication (days)



Pie diagram showing most of the ulcers (43.3%) were free from biofilm by 10-15 days.

Table 12 : Mean Distribution of Eradication (days)

	Minimum	Maximum	Mean	SD
ERADICATION (days)	10	25	18.1	5.0

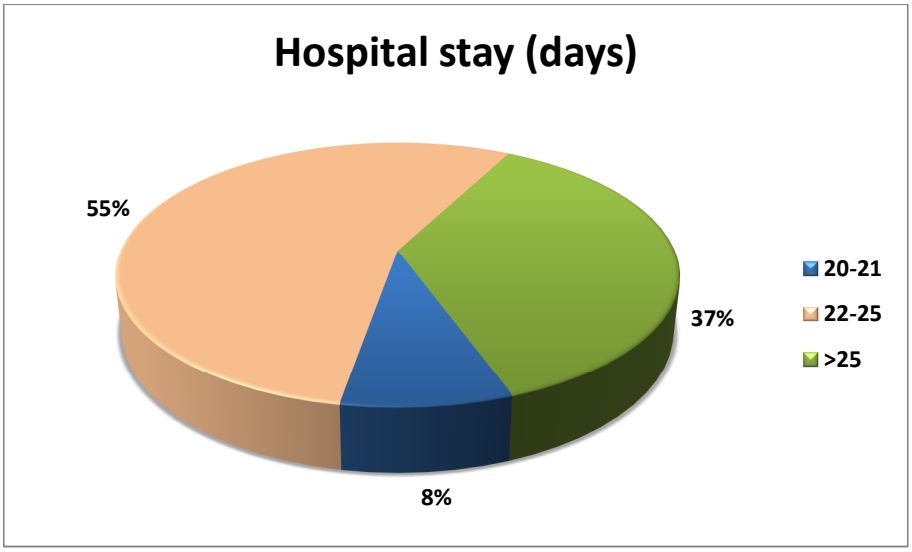
The mean duration for eradication of biofilm in this study was 18.1+/-5.0 days.

Table 13 : Percent Distribution of Hospital stay (days)

Hospital stay (days)	N	Percent
20-21	5	8.3
22-25	33	55
>25	22	36.7
Total	60	100

The duration of hospital stay was 22-25 days in 55% of patients, 20-21 days in 8.3% of patients and more than 25 days in 36.7% of patients.

GRAPH 9 : Percent Distribution of Hospital stay (days)



Pie diagram depicting maximum patients (55%) with hospitalisation of 22-25 days.

Table 14 : Mean Distribution of Hospital stay (days)

	Minimum	Maximum	Mean	SD
HOSPITAL STAY (days)	20	30	26.4	3.1

The mean duration of hospital stay in this study was 26.4+/-3.1 days .

Table 15 : Association of BIOFILM and Age (Yrs)

BIOFILM	21-30		31-45		46-65		>65		Total		p value
	N	%	N	%	N	%	N	%	N	%	
EC	0	0	1	5.9	3	11.5	4	33.3	8	13.3	0.011
EF	0	0	0	0	0	0	1	8.3	1	1.7	
KO	1	20	0	0	0	0	0	0	1	1.7	
KP	1	20	0	0	1	3.8	0	0	2	3.3	
KP,EC	0	0	1	5.9	0	0	0	0	1	1.7	
PA	0	0	5	29.4	4	15.4	0	0	9	15	
SA	3	60	10	58.8	17	65.4	5	41.7	35	58.3	
SA,EC	0	0	0	0	0	0	2	16.7	2	3.3	
SA,PA	0	0	0	0	1	3.8	0	0	1	1.7	
Total	5	100	17	100	26	100	12	100	60	100	

Table 16 : Association of BIOFILM and DIAGNOSIS

BIOFILM	LFNHU		LLNHU		RFNHU		RHNHU		RLNHU		Total		p value
	N	%	N	%	N	%	N	%	N	%	N	%	
EC	3	30	2	11.8	2	16.7	0	0	1	5.9	8	13.3	0.34
EF	0	0	0	0	1	8.3	0	0	0	0	1	1.7	
KO	0	0	1	5.9	0	0	0	0	0	0	1	1.7	
KP	1	10	1	5.9	0	0	0	0	0	0	2	3.3	
KP,EC	0	0	0	0	1	8.3	0	0	0	0	1	1.7	
PA	2	20	0	0	0	0	1	25	6	35.3	9	15	
SA	4	40	10	58.8	8	66.7	3	75	10	58.8	35	58.3	
SA,EC	0	0	2	11.8	0	0	0	0	0	0	2	3.3	
SA,PA	0	0	1	5.9	0	0	0	0	0	0	1	1.7	
Total	10	100	17	100	12	100	4	100	17	100	60	100	

Table 17 : Association of BIOFILM and ACUTE/CHRONIC

BIOFILM	ACUTE		CHRONIC		Total		p value
	N	%	N	%	N	%	
EC	1	25	7	12.5	8	13.3	0.985
EF	0	0	1	1.8	1	1.7	
KO	0	0	1	1.8	1	1.7	
KP	0	0	2	3.6	2	3.3	
KP,EC	0	0	1	1.8	1	1.7	
PA	0	0	9	16.1	9	15	
SA	3	75	32	57.1	35	58.3	
SA,EC	0	0	2	3.6	2	3.3	
SA,PA	0	0	1	1.8	1	1.7	
Total	4	100	56	100	60	100	

Table 18 : Association of BIOFILM and Hospital stay (days)

BIOFILM	20-21		22-25		>25		Total		p value
	N	%	N	%	N	%	N	%	
EC	0	0	5	15.2	3	13.6	8	13.3	0.874
EF	0	0	0	0	1	4.5	1	1.7	
KO	0	0	1	3	0	0	1	1.7	
KP	0	0	2	6.1	0	0	2	3.3	
KP,EC	0	0	1	3	0	0	1	1.7	
PA	2	40	3	9.1	4	18.2	9	15	
SA	3	60	19	57.6	13	59.1	35	58.3	
SA,EC	0	0	1	3	1	4.5	2	3.3	
SA,PA	0	0	1	3	0	0	1	1.7	
Total	5	100	33	100	22	100	60	100	

Table 19 : Association of BIOFILM and Eradication (days)

BIOFILM	10-15		16-20		>20		Total		p value
	N	%	N	%	N	%	N	%	
EC	2	7.7	2	9.5	4	30.8	8	13.3	0.517
EF	0	0	1	4.8	0	0	1	1.7	
KO	1	3.8	0	0	0	0	1	1.7	
KP	0	0	1	4.8	1	7.7	2	3.3	
KP,EC	1	3.8	0	0	0	0	1	1.7	
PA	3	11.5	5	23.8	1	7.7	9	15	
SA	17	65.4	12	57.1	6	46.2	35	58.3	
SA,EC	1	3.8	0	0	1	7.7	2	3.3	
SA,PA	1	3.8	0	0	0	0	1	1.7	
Total	26	100	21	100	13	100	60	100	

Table 20 : Association of BIOFILM and STSG (days)

BIOFILM	10-15		16-20		>20		Total		p value
	N	%	N	%	N	%	N	%	
EC	5	15.2	2	12.5	1	9.1	8	13.3	0.519
EF	0	0	0	0	1	9.1	1	1.7	
KO	1	3	0	0	0	0	1	1.7	
KP	1	3	0	0	1	9.1	2	3.3	
KP,EC	0	0	0	0	1	9.1	1	1.7	
PA	5	15.2	3	18.8	1	9.1	9	15	
SA	19	57.6	11	68.8	5	45.5	35	58.3	
SA,EC	1	3	0	0	1	9.1	2	3.3	
SA,PA	1	3	0	0	0	0	1	1.7	
Total	33	100	16	100	11	100	60	100	

Table 21 : Association of BIOFILM and GRANULATION TISSUE (days)

BIOFILM	10-15		16-20		21-30		Total		p value
	N	%	N	%	N	%	N	%	
EC	2	7.7	3	15	3	21.4	8	13.3	0.121
EF	0	0	1	5	0	0	1	1.7	
KO	1	3.8	0	0	0	0	1	1.7	
KP	2	7.7	0	0	0	0	2	3.3	
KP,EC	0	0	0	0	1	7.1	1	1.7	
PA	3	11.5	4	20	2	14.3	9	15	
SA	18	69.2	12	60	5	35.7	35	58.3	
SA,EC	0	0	0	0	2	14.3	2	3.3	
SA,PA	0	0	0	0	1	7.1	1	1.7	
Total	26	100	20	100	14	100	60	100	

DISCUSSION

In the context of the continued emergence of antibiotic resistant pathogens, some alternative or "traditional" topical antimicrobials have been reintroduced into modern wound care, one such example being honey. Although the microflora of chronic wounds is polymicrobial and heterogeneous, *S. aureus* and *P. aeruginosa* are among the bacteria that are most frequently isolated from the wounds in our study.

This study presents a comprehensive clinical and microbiological survey of biofilms isolated from acute and chronic ulcers of the patients admitted to our hospital. We took culture samples from the 60 wounds with results showing predominantly Staphylococcus aureus (58.3%), Pseudomonas aeruginosa (15%), Escherichia coli (13.3%), Klebsiella pneumonia(3.3%),Enterococcus fecalis (1.7%), Klebsiella oxytoca (1.7%) and polymicrobial (6.7%). Whereas in other study by SR. Swarna and etal[83] with a sample size of 62, showed S.aureus (29.26%), E.coli (19.51%), P. aeruginosa (19.51%), K.pneumoniae (4.87%), Proteus species (4.87%), Acinitobacter species (4.87%), Citrobacter species (2.43%).

In our study the incidence of biofilm in acute ulcers was 6.7% and in chronic wounds was 93.3% and with study done by James GA et al[3], where the incidence of biofilm was 6% in acute ulcers and 60% in chronic ulcers and compared to study done by SR Swarna et al[83], where the incidence of biofilm was 70% in chronic ulcers and 6% in acute ulcers.

In our study the most common organism isolated was Staphylococcus aureus followed by Pseudomonas aeruginosa and is consistent with the study in 2010 by Thomsen TR et al[84], where the most common organism isolated was staphylococcus

aureus. S.aureus and P. aeruginosa are the most frequent isolated bacteria from these wounds.

The age group in our study is 21-90 years and is comparable with the studies done by SR Swarna et al[83], where the age group was 30 to 80 years.

In our study, the mean duration of hospital stay of ulcers with biofilm is 26.4±3.1 days and is consistent with the similar study done by Zhao G et al[85], which showed a mean duration of hospital stay was 28 ± -4 days.

This study showed reduction in the size of ulcer and formation of granulation tissue by 18.1±5.5 days whereas in a study by Subramanyam M,[86] showed Honey dressing significantly stimulated the rate of burn wound healing as demonstrated by formation of granulation tissue and reduction in wound size especially after 21 and 28 days after burn, wheras in another study by H. Maghsoudi[87] et al, showed Clinical evidence of granulation tissue formation and epithelialization of raw areas were observed in comparative study between 42 patients in honey group and 36 patients in Mafenide acetate group by day 7. In honey-treated patients, all the wounds healed by day 21 (100%) compared to 42 patients (84%) ($p < 0.001$) in the mafenide acetate treated group.

The mean duration of wound contraction and reduction in size of ulcer with formation of granulation tissue is 18±5.5 days , whereas in a study by Mui Koon Tan et[88] al The Efficacy of Gelam Honey Dressing towards Excisional Wound Healing , comparative study of treating ulcers with honey, saline and no treatment , showed that the contraction of wound area was significantly increased with wound contraction in honey group significantly ($P < 0.05$) greater compared to untreated group and saline group by 15 days .

The mean duration of hospital stay in our study is 26.4 ± 3.1 days whereas in a study H.Maghsoudi[87] et al in 2011, Comparison between topical honey and mafenide acetate in treatment of burn wounds the mean hospital stay in the honey-treated group was 22 ± 1.2 days versus 32.3 ± 2 days in the mafenide acetate group ($p < 0.005$, significant).

Biofilms, significantly increase the ability of the pathogen to evade both host defenses and antibiotics. They are being implicated in the pathogenesis and also clinical manifestation of several infections. They cause a variety of persistent infections, including chronic middle ear infections, bone infections, heart valve infections, infections related to implanted medical devices, and lung infections in people with the autosomal recessive inherited disease like cystic fibrosis. The chronic nature of some urinary tract infections is being attributed to the ability of *Escherichia coli* to form a biofilm.

SUMMARY

- In our study of Management of Biofilms in acute and chronic ulcers with local application of honey, we found that out of total 60 patients mean age was of 54.5 ± 16.6 in years.
- Highest number of patients were in the age group of 45-65 years, 26 patients – 43.3%.
- Amongst 60patients, 50 were males and 10 females.
- Out of 60 ulcers, 56 were chronic ulcers and 4 were acute ulcers.
- In 60 ulcers with biofilm, staphylococcus aureus was present predominantly in 58.3% patients.
- The mean duration for eradication of biofilm in this study was 18.1 ± 5.0 days.
- The mean duration for formation of healthy granulation tissue in this study was 18.1 ± 5.5 days.
- The mean duration for STSG in this study was 16.9 ± 4.4 days.
- All patients were discharged with a mean duration time of 26.4 ± 3.1 days.
- In 3 patients, biofilm of multiple organisms was present. 3.3% patients had SA and EC, 1.7% patients had PA and SA.

CONCLUSION

- All patients admitted for acute and chronic ulcers with biofilm were effectively managed by local application of honey with significant reduction in the hospital stay.
- There were no side effects or reactions in any patients except pain at application site because of low pH of honey.

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ANNEXURES

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 13-11-2013 at 3-30pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "Management of biofilms in acute and chronic ulcers by local application of Honey" —x—x—

Name of P.G. student Dr Anand Sagar Ragate
Department of Surgery.

Name of Guide/Co-investigator Dr Tejaswini Vallabha.
Prof & H.O.D. of Surgery

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

SAMPLE INFORMED CONSENT FORM

B.L.D.E.U's SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR– 586103, KARNATAKA.

TITLE OF THE PROJECT	:	MANAGEMENT OF BIOFILMS IN ACUTE AND CHRONIC ULCERS BY LOCAL APPLICATION OF HONEY.
PRINCIPAL INVESTEGATOR	:	Dr. ANAND SAGAR RAGATE Department of General Surgery Email:asrthedoc@gmail.com
PG GUIDE	:	Dr. TEJASWINI VALLABHA M.S. Professor& HOD General Surgery B.L.D.E. University's Shri B.M. Patil Medical College Hospital & Research Centre, Sholapur Road, VIJAYAPUR - 586103

PURPOSE OF RESEARCH:

I have been informed that this study will analyze MANAGEMENT OF BIOFILMS IN ACUTE AND CHRONIC ULCERS WITH LOCAL APPLICATION OF HONEY..

I have been explained about the reason for doing this study and selecting me/my ward as a subject for this study. I have also been given free choice for either being included or not in the study.

PROCEDURE:

I understand that relevant history will be taken. I will undergo detailed clinical examination after which necessary investigations will be done whenever required, which would help the investigator for appropriate management.

RISKS AND DISCOMFORTS:

I understand that I/my ward may experience some pain and discomfort during the examination or during my treatment. This is mainly the result of my condition and the procedure of this study is not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that I/my wards participation in this study will help to analyse the effectiveness of n butyl cyanoacrylate glue in reducing post-operative pain and complications.

CONFIDENTIALITY:

I understand that medical information produced by this study will become a part of this Hospital records and will be subjected to the confidentiality and privacy regulation of this hospital. Information of a sensitive, personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secure location.

If the data are used for publication in the medical literature or for teaching purpose, no names will be used and other identifiers such as photographs and audio or video tapes will be used only with my special written permission. I understand that I

may see the photograph and videotapes and hear audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Dr. ANAND SAGAR RAGATE is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which might influence my continued participation.

If during this study or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me and that a copy of this consent form will be given to me for careful reading.

REFUSAL OR WITHDRAWL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital.

I also understand that Dr. ANAND SAGAR RAGATE will terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician or therapist, if this is appropriate.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me/my ward, resulting directly to my participation in this study, if such injury were reported promptly, then medical treatment would be available to me, but no further compensation will be provided.

I understand that by my agreement to participate in this study, I am not waiving any of my legal rights.

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits, to the best of my ability in patient's own language.

Date:

Dr. TEJAWINI VALLABHA (Guide)

Dr. ANAND SAGAR RAGATE (Investigator)

STUDY SUBJECT CONSENT STATEMENT:

I confirm that Dr. ANAND SAGAR RAGATE has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give my consent to participate as a subject in this research project.

_____

(Participant) Date

_____

(Witness to above signature) Date

PROFORMA FOR CASE TAKING

SL NO:	IP NO:
Name:	UNIT:-
Age/Sex:	DOA:-
Religion:	DOS:-
Occupation:	DOD:-
Address:	

Chief complaints**:**

History of presenting complaints**:**

Past history:

Comorbidities:

PERSONAL HISTORY:

Diet:	Appetite:	Bowel/Bladder:
Sleep:	Digestion:	Habits:

GENERAL PHYSICAL EXAMINATION:

Built: Well/Moderate/Poor

Nourishment: Well/Moderate/Poor

Pallor/Icterus/Cyanosis/clubbing/pedal oedema/ lymphadenopathy

BP: PR: RR:

Temperature: SPo_2:

SYSTEMIC EXAMINATION:

Per Abdomen:

Respiratory System:

Cardio Vascular System:

Central Nervous System:

Local examination of wound:

LABORATORY TESTS

Haemoglobin% : BT: CT:

Total Count : N L E B M

Platelets :

Blood Urea :

Serum Creatinine :

HIV : HB_SAg:

Electro Cardiogram:

Urine routine :

X- RAY of effected part:

Pus for GramStain: Pus for C/S:

Presence of Biofilm: Yes/No

FINAL DIAGNOSIS :

TYPE OF DRESSING:HONEY/DEBRIDEMENT

OBSERVATION OF THE WOUND WITH BIOFILM:

VARIABLES	**DAY 5**	**DAY 10**	**DAY 15**	**DAY 20**	**DAY 25**	**DAY 30**
FOUL SMELL						
DISCHARGE						
GRANULATION TISSUE						
SIZE OF THE ULCER						

Interventions done for the wound:

- Skin grafting:
- Delayed primary closure:
- Secondary suturing:
- Time for healing of wounds with biofilm:

INFERENCE:

REMARKS:

KEY TO MASTER CHART

IP.NO	-	IN PATIENT NUMBER
DOA	-	DATE OF ADMISSION
DOS	-	DATE OF SURGERY
DOD	-	DATE OF DISCHARGE
STSG	-	SPLIT THICKNESS SKIN GRAFTING
EC	-	ESCHERICHIA COLI
EF	-	ENTEROCOCCUS FECALIS
KO	-	KLEBSIELLA OXYTOCA
KP	-	KLEBSIELLA PNEUMONIAE
SA	-	STAPHYLOCOCCUS AUREUS
LFNHU	-	LEFT FOOT NONHEALING ULCER
LLNHU	-	LEFT LEG NONHEALING ULCER
RHNHU	-	RIGHT HAND NONHEALING ULCER
RFNHU	-	RIGHT FOOT NONHEALING ULCER
RLNHU	-	RIGHT LEG NONHEALING ULCE