1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The wound is a breach in the cellular, anatomic and functional continuousness of living tissues which may result not only due to the external factors but also as a complication of some diseases (Ayello, 2005).

Every living tissue responds to injury. Tissue disruption, be it operative or traumatic, instigates a set of priorities in every organism. They include termination of bleeding, avoiding infection and refurbishment of the integrity and function of the tissue. This process of tissue repair is called wound healing.

Wound healing is a process that commences with an injury and usually terminates in the formation of a scar tissue (Rubin and Farber, 1996). Traditionally, it is divided into four stages: hemostasis, inflammation, proliferation, and remodeling (Figure 1.1). Further, the wound healing also involves different phases such as coagulation, epithelization, granulation, collagenation, and tissue remodeling (Fulzele et al., 2002).

![Figure 1.1: The phases of Wound Healing. Source: http://www.shieldhealthcare.com](image-url)
Wound healing has three major stages: An inflammatory phase wherein cytokines/growth factors and other inflammatory mediators are produced (Hunt et al., 1999). The Reactive oxygen species (ROS) are also secreted in this phase, which in low levels is vital to protect the wound from the invading bacteria and other microorganisms (Schäfer and Werner, 2008). However, overexposure to ROS causes oxidative stress resulting in delayed wound healing (Mizuta et al., 2012). The next phase of healing is the proliferation phase wherein cells like fibroblasts and endothelial cells are recruited. The last step includes the generation and reorganization of extracellular matrix (ECM) resulting in tissue repair and regeneration (Hunt et al., 1999).

Various factors influence healing of a wound. Diet, hygiene, external environment, and infection may externally affect wound healing. The internal factors that affect wound healing include regulators like growth factors, cytokines, and physiological condition of the individual (Ayello, 2005).

The management of the wound is therefore complicated especially when the wound is of a non-healing type. The most common type of non-healing wounds are the diabetic wounds. Diabetic wounds, unlike typical wounds, heal slowly resulting in treatment with conventional topical medicines a challenging and painstaking process. The regulators of wound healing become dysfunctional in diabetic wounds which lead to a delay in the refurbishment. (Brem and Tomic-Canic, 2007).

Wounds have affected humans since pre-historic times, and the treatment of healing wounds is an art which is as old as humanity (Robson et al., 2001). Research on the
drugs helpful in wound healing is a rapidly growing area in biomedical sciences. The advancement in this field has enabled the production of numerous molecules related with the process of wound repair. However, the current trends in the wound management targets only to avoid inflammation and growth of microbes at the site of the injury (Robson et al., 2001).

Although modern medicine has found advancement in the management of the wounds, poor wound management is still observed. This problem is attributed to unawareness, indecorous hygiene, the absence of essential health care needs and remoteness of the health centers.

Ayurveda and folk medicinal systems, on the other hand, are readily available and encompass many entities that traditionally accelerate healing. Healers yearn for traditional medicines for their high acceptability rate and real toleration (Jagetia et al., 2003).

Therefore, the alternative medicinal systems such as Ayurveda and Folk Medicine are preferred for the treatment and wound management. Amidst many effective treatment strategies are the use of Honey, Ghee and Glycyrrhiza glabra (GG) and a folk medicinal preparation using Nerium indicum (NI). They are efficiently utilized in the treatment of different types of wounds since many years in India. However, the exact biomechanical, cellular, biochemical and molecular mechanisms underlying this traditional medicinal preparation mediated wound healing are less explored.
1.1.1. Definition of the problem

The wound management in the current scenario involves medications that only aims to avoid inflammation and growth of microbes in the wound. As wound healing is a complex process and is influenced by various extrinsic and intrinsic factors, therefore the management of chronic wounds become medically challenging. In the recent years, the use of traditional medicines in the treatment of such injuries is in practice. This practice is because the traditional polymolecular medicine has many advantageous effects compared to a single molecule based allopathic medicine on several occasions. The Indian traditional medicinal systems such as Ayurveda and folk medicine have been endlessly utilizing active medicinal formulations in the treatment of wounds. They have claimed that these medicinal preparations are not only anti-inflammatory, antimicrobial but also possess regenerating and rejuvenating effects (Datta et al., 2011).

Several studies have identified the wound healing benefits of individual application of Honey (Jull et al., 2008) and Ghee (Prasad and Dorle, 2006). However, they focused on only the results such as faster healing, wound closure, increased hydroxyproline content, etc. Further, there are hardly any studies that signify the combined effect of Honey and Ghee. GG (Licorice) is also known for its wound healing properties in Ayurveda. Studies have evidenced the wound healing, anti-inflammatory, antiulcer, and skin regeneration activity of Licorice (Oloumi et al., 2007). NI is another herb employed in the folk medicine to treat wounds (Sravanthi et al., 2010). However, there are rarely any studies that have been carried out to provide an explanation for the exact cellular, biochemical and molecular mechanisms involved in wound healing by these medicines. Further, the involvement of these traditional medicines in the treatment of chronic wounds such as diabetic wounds is less explored.
Therefore, the present study intends to scientifically evaluate the fundamental mechanisms of wound healing by the topical application of these medicines singly and in combination. We wish to study the healing effects on both normal (representative of the acute wound) and diabetic groups (representative of the chronic wound). The study may also provide an understanding of the actual healing process, the mechanisms of which otherwise remains elusive.
1.2. Literature review

A wound is described as a consequence of injury to the epithelial surface and associated tissues. It could be aggravated by the reduced tissue perfusion and inadequate oxygenation.

Wounds are classified into two broad categories: Acute and Chronic. Acute wounds typically heal by the phases of hemostasis and inflammation. The healing is then followed by tissue repair and regeneration that occurs within a stipulated time of 30 days approximate. Conversely, chronic wounds, fail to heal within the required time due to the interruption in the wound healing cascade and associated pathologies, mainly infection (Cohen et al., 1999).

The rise in the cardiovascular and neurological diseases like diabetes in our aging population increases the number of patients getting affected by chronic and complicated wounds (Gosain and DiPietro, 2004; Guo and DiPietro, 2010). Effective wound management is therefore imperative to decrease the rates of mortality and morbidity. It is also useful to lessen the exhaustion of hospital resources and the increased expenses associated with this increasing health problem (Sen et al., 2009; Posnett and Franks, 2008).

1.2.1. The process of wound healing

The process of healing in an acute skin injury is described below.

The acute skin wounds can be of different types, i.e., incisional, partial thickness wounds, and injuries involving substantial loss of tissue. Although the wounds are of various kinds, the phases of healing, however, remains the same with varying degrees.
1.2.2. Stages of wound healing

The sequence of acute wound healing occurs in a carefully regulated manner. It follows a predictable series of events. The sequences of wound healing typically overlap. However, for a clear understanding, they are explained below in a step-wise manner. The five stages of the wound healing process are hemostasis, inflammation, cellular migration & proliferation, protein synthesis & wound contraction, and remodeling (Monaco and Lawrence, 2003) (Figure 1.2).

![Figure 1.2: The stages of Cutaneous Wound Healing (Beanes et al., 2003)](image)

1.2.2.1. Hemostasis

Wounds result in a vascular injury that further activates the molecular and cellular responses thereby establishing hemostasis. The healing process cannot continue until hemostasis is obtained. Vasoconstriction, platelet aggregation, fibrin deposition and
1.2.2.1.1. Vasoconstriction

The vasoactive amines released on dermal penetration leads to vasoconstriction. Epinephrine is liberated into the peripheral circulation. The local norepinephrine also gets released by the activation of the sympathetic nervous system. Prostaglandins, such as thromboxane secreted by the injured cells further contribute to vasoconstriction (Monaco and Lawrence, 2003).

1.2.2.1.2. Platelet aggregation

Platelet aggregation is caused by the contact with the tissue factors secreted by damaged cells. Immediately after the injury, the sequence of healing gets initiated when the platelets encounter the exposed collagen (Figure 3). As the aggregation of the platelets continues, clotting factors are secreted leading to the fibrin clot formation at the wound site. The fibrin clot acts as a provisional matrix and provides a platform for the successive healing events (Clark, 2001). Platelets in addition to releasing the clotting factors also yield cytokines/ growth factors that stimulate the process of healing (Diegelmann and Evans, 2004).

1.2.2.1.3. Clot Formation

Clot formation is the end product of the hemostasis. A meshwork of fibrin, aggregated platelets along with the blood cells embedded within it forms a clot (Lawrence, 1998). The clot formation is of paramount significance. This process avoids further loss of fluid and electrolytes from the wound area and prevents infection. Fibrin also serves
as a framework in the interim wound matrix allowing the migration of the fibroblasts and other cells onto it during the further stages of repair (Monaco and Lawrence, 2003).

Insufficient fibrin deposition results in a bad wound. It ruins cell adhesion and chemotaxis (Fukai et al., 1991). Removal of fibrin from the wound disturbs the formation of ECM thereby resulting in delayed wound healing (Clark et al., 1990).

1.2.2.2. Inflammation

Inflammation is characterized by the rise in the permeability of the blood vessels and the subsequent passage of leukocytes into the ECM (Cohen et al., 1999). Inflammation is primarily involved in attracting the inflammatory cells to the wound site (Kloth et al., 1990). These cells abolish the bacteria and remove debris from the injured matrix thereby aiding in the process of healing (Majno et al., 1969).

Although inflammation is usually regarded as the second stage of wound repair, the inflammatory changes arise soon after injury due to vasodilatation (Monaco and Lawrence, 2003).

1.2.2.2.1. Inflammatory Cells

**Neutrophils:** They are the principal cells at the wound site, and appear within 24 hours following wounding. They help to eliminate foreign particles, bacteria, useless cells and impaired constituents of the matrix available at the site of injury (Hart, 2002; Sylvia, 2003). Neutrophils are attracted by the chemical signals conveyed by the bacteria that result in the bacterial ingestion by phagocytosis. During this process,
f-Met-Leu-Phe, a tripeptide is released which entices the cells of inflammation (Tschaikowsky et al., 1993). Neutrophils expand by accumulating the bacteria and forms a "laudable pus" at the wound site (Thurston, 2000).

**Mast Cells:** They secrete granules packed with enzymes such as histamine and other active amines at the wound site which results in the characteristic inflammatory signs (Artuc et al., 1999). The active amines secreted by the mast cell makes the vessels surrounding them permeable thereby enabling faster conveyance of the mononuclear cells into the wound site. Also, fluid collects in the area of injury, and the inflammatory signs begin. The inflammatory signs include rubor (redness), calor (heat), dolor (pain) and tumor (swelling) (Diegelmann and Evans, 2004).

**Monocyte & Macrophages:** 48 hours post wounding, monocytes present at the site of injury get triggered to form wound macrophages. The latter is the important cell of inflammation responsible for the normal healing (Diegelmann et al., 1981). Their inhibition delays the responses of healing (Leibovich and Ross, 1975). Activated wound macrophages also secrete growth factors like PDGF (platelet-derived growth factor) and TGF-ß (transforming growth factor- beta) that entices the fibroblasts and smooth muscle cells (SMCs) to the wound site. The macrophages are also phagocytic and are involved in the elimination of non-functional cells, neutrophils filled with bacteria, injured matrix, extraneous particles, etc. from the site of injury. The existence of macrophages at the location of wound indicates the termination of the phase of inflammation and initiation of the phase of proliferation (Diegelmann and Evans, 2004).
**Lymphocytes:** Lymphocytes are observed at the wound site at a later stage. Although their exact role in the wound healing process is uncertain, however, it has been stated that T lymphocytes play a critical role in wound healing and the healing cascade gets inhibited by the removal of circulating T lymphocytes (Peterson et al., 1987). B lymphocytes, on the contrary, do not have any significant part in wound repair (Martin and Muir, 1990). Classically, both CD4 and CD8-positive T lymphocytes are available in highest levels on fifth to seventh-day post wounding. This increased concentration is influenced by IL-2 (interleukin-2) and many other factors of immunomodulation (Nielson et al., 1982; Witte and Barbul, 1997). The CD4-positive T lymphocytes release cytokines such as IL-1, IL-2, TNF-α (tumor necrosis factor-alpha), fibroblast activating factor, EGF (epidermal growth factor), and TGF-β (Minchenko et al., 1994; Patel et al., 1994; Schreiber et al., 1986). T cells are also responsible for cell-mediated immunity (Fauci and Haynes, 1994).

**Eosinophils and Basophils:** Eosinophils and basophils are the other inflammatory cells observed at the wound site. Uncontrolled accumulation of eosinophils and basophils at the site of injury results in the damage of the host tissue (Martin and Muir, 1990). These cells attain maximum levels at 24-48 hours post wounding and can produce TGF-α (Peterson et al., 1987).

As the wound healing continues, inflammatory cells imprisoned inside the clots are removed (Gailit and Clark, 1994). The factor responsible for the apoptosis of the inflammatory cells during healing and scar formation is undetermined. Neutrophils are the first among the inflammatory cells to undergo senescence and apoptosis (Simpson and Ross, 1972). They are then phagocytosed by macrophages (Newman et
al., 1982). The macrophages and lymphocytes persist in the site of injury for nearly seven days and eventually reduce in number unless a harmful irritant causing further inflammation perseveres. Apoptosis of the inflammatory cells impact the presentation of antigens and mainly leads to the variation in the levels of cytokines (Cohen et al., 1993).

1.2.2.2. Cytokines/ Growth Factors

Post-injury, proinflammatory cytokines act as major modulators of the process of inflammation. The two primary signals are PDGF and TGF-β (Kim et al., 1998). These signals play a significant role in the later phases of healing. The PDGF is responsible for the migration of neutrophils, macrophages, SMCs and fibroblasts towards the site of injury. It also increases the number of fibroblasts and SMCs. TGF-β is involved in the commencement of the events of healing by enticing the macrophages and further stimulating them to release extra growth factors such as IL-1, PDGF, TNFα and FGF (fibroblast growth factor). Also, TGF-β increases the chemotaxis of fibroblasts and SMCs and modulates the activity of collagen and collagenase (Diegelmann and Evans, 2004).

IL-1 is a major “alarm” / pro-inflammatory cytokine derived mainly from the platelets and macrophages. It acts through the stimulation of a network of chemokines, cytokines, and other small molecule mediators. It also causes the leukocytes, endothelial and other cells to express adhesion molecules and integrins (Dinarello, 1996; Dinarello, 2009; Apte et al., 2006; Apte and Voronov, 2008). It enables cell infiltration, inflammation and tissue repair at the site of damage.
EGF act as a mitogen for fibroblasts and keratinocytes. It helps in the formation of the granulation tissue and migration of the keratinocytes. FGF 1 and 2, helps in the fibroblast chemotaxis & proliferation, keratinocyte proliferation, angiogenesis, wound contraction and matrix deposition (Diegelmann and Evans, 2004). VEGF (vascular endothelial growth factor) on the other hand acts as an endothelial cell mitogen (Leung et al., 1989), chemotactic agent (Yoshida et al., 1996; Noiri et al., 1998) and inducer of vascular permeability (Senger et al., 1990; Ferrara, 1999). VEGF is exclusive in its influence on numerous wound healing mechanisms, such as angiogenesis, epithelization and collagen deposition (Stojadinovic et al., 2007) (Figure 1.3).

Figure 1.3: Cytokines/ Growth Factors in Wound Healing

(Demidova-Rice et al., 2012)
The cytokines/growth factors are thus responsible for a dynamic response of the cellsproducing matrix and ensuring quick deposition of newly formed connective tissue at the site of the wound in the phase of proliferation (Diegelmann and Evans, 2004).

1.2.2.3. Cellular Migration and Proliferation

The cell composition of the wound undergoes profound changes in the first week after injury. The inflammatory cells that initially populated the fibrin–fibronectin matrix is soon replaced by the fibroblasts, keratinocytes and endothelial cells as healing advances.

1.2.2.3.1. Fibroblasts in Wound Healing

Fibroblasts are the most abundant cells in the dermis and entirely accountable for the production of most of the ECM in the dermis. Fibroblasts have a prime role in healthy wound healing. They are often associated with macromolecules and reside onto the network of ECM. Bioactive cells are called fibroblasts, and when they differentiate into inactive cells, they are termed fibrocytes. The function of fibrocytes is entirely restricted to the synthesis of ECM such as collagen, elastin, hyaluronic acids, and glycosaminoglycans, whereas fibroblasts participate in the regenerative processes by actively synthesizing ECM and responding to wound healing or inflammatory conditions. Fibroblasts retain the capacity to differentiate into myofibroblasts, smooth muscle cells, chondrocytes and osteocytes upon stimulation. Fibroblasts modulate the tissue homeostasis by producing and degrading ECM and providing growth factors. It is significant in wound healing wherein activated fibroblasts migrate to the area, proliferate and synthesize new ECM like collagen and elastin to produce granulation tissue. After the maturation of collagen, the wound contracts with the help of enzymes secreted from the fibroblasts (Grinnell, 1994).
In the skin, collagen, fibronectin, and laminins play key roles as they control the composition of the basal lamina and influence the dermal-epidermal interactions. Dermal fibroblasts are also involved in the formation of the epidermis by producing a diffusible factor which modulates the epidermal proliferation and survival (Coulomb et al., 1989).

1.2.2.3.2. Keratinocytes in Wound Healing

Keratinocytes constitute the major cellular component in the epidermis and play critical roles in the process of wound healing. They play a major part in the complex mechanisms involved in the initiation, continuation, and completion of wound healing. The properties of keratinocytes in chronic wounds differ in different locations and situations. Normally keratinocytes are responsible for the proliferative and regenerative capacity of the epidermis by providing a continuous supply of proliferative cells. The re-epithelization of the wounded skin occurs by the rapid and coordinated migration of keratinocytes.

The keratinocytes present at the edges of the non-healing chronic wounds are different from the healthy and normal keratinocytes. A successful wound closure requires a cross-talk between the keratinocytes and other cell types involved in wound healing (Paster et al., 2008). Keratinocytes may also play a role in the ECM reorganization (Isaac et al., 2011).

Restoration of the denuded epithelial surface and revascularization of the damaged area begins during the proliferative phase. Cytokines remain an integral part of this process. It is because their expression is essential for the events like epithelialization,
fibroplasia, and angiogenesis (Fauci and Haynes, 1996). Although adequate information is available regarding the indicators that activate the major steps during this healing phase, however the information about the signs that terminate these steps are inadequate. Deactivation of the cells by negative feedback mechanisms on successful completion of their assigned tasks are also imperative for effective wound healing (Monaco and Lawrence, 2003).

The fibroblasts begin to repopulate the site of the wound by migration and proliferation during this phase. Additionally, under the effect of the growth factors, the undifferentiated cells present around the site of injury can also convert into fibroblasts (Postlethwaite et al., 1987).

With the advancement of the phase of proliferation, the TGF-ß expressed by the platelets, macrophages and T lymphocytes develop as a vital signal. TGF-ß is regarded as a significant indicator that modulates multiple fibroblast functions (Roberts and Sporn, 1993). It has a triple influence on the deposition of ECM (Roberts et al., 1992): 1) Increases the overall production of proteins of the ECM matrix by increasing the gene transcription for collagen, proteoglycans, and fibronectin. 2) Reduces the release of proteases that are liable for the damage to the matrix. 3) It also stimulates tissue inhibitor of metalloprotease (TIMP), the protease inhibitor (Hall et al., 2003). Other cytokines important during this phase include interleukins, FGFs and TNF-α (Diegelmann and Evans, 2004).

As the healing advances, many other significant biological activities are instigated such as epithelization & angiogenesis.
1.2.2.3.3. Epithelization

The reconstruction of the disrupted epithelium after an acute injury is essential for the restoration of the function of the skin as a barrier. Refurbishment of damaged epithelium begins soon after injury. The skin incision wounds, with a slight gap in the epithelium, are classically re-epithelialized within 24-48 hours following wounding (Lawrence, 1998; Nanney et al., 1984). But larger wounds may take a longer time for re-epithelization. In the first 24 hours following wounding, the basal cells occupying the edges of the wound prolong and moves towards the damaged wound area. The appendages of the skin such as sweat glands and hair follicles which when spared during the formation of the wound, also provide epithelial cells that migrate to the wound site. These cells drift towards the injured surface and are commonly arranged as a monolayer. More basal cells from the wound edges and appendages migrate to this area, nearly after 24 hours of origination of the cell migration and begin to proliferate, thereby adding to the healing monolayer. This process of migration endures until an overlap is attained among the epithelial cells drifting from diverse ways. Ones the overlap is achieved, the cellular migration stops due to “contact inhibition” (Monaco and Lawrence, 2003).

The re-epithelization is roused by the expression of EGF, TGF-α and Keratinocyte growth factor (KGF) that are generated by wound macrophages, platelets and keratinocytes (Yates et al., 1991; Schultz et al., 1991; Hunt et al., 1984). The migration of the cells can also be influenced by the release of matrix metalloproteinases (MMPs) which enter the eschar or scab (Vu and Werb, 2000). On the completion of the epithelization, certain enzymes are secreted that loosens the connection of the scab at its base leading to its detachment. (Diegelmann and Evans, 2004).
1.2.2.3.4. Angiogenesis

Increased metabolic activity at the site of the wound leads to a growing need for oxygen and nutrients. The conditions in the microenvironment of the wound such as reduced pH, decreased oxygen tension, and higher levels of lactate lead to the release of factors that regenerate the blood supply (LaVan and Hunt, 1990; Knighton et al., 1983). This process is termed neovascularization/angiogenesis and is caused by cytokines such as VEGF, FGF, and TGF-β (Tonnesen and Clark, 2000; Battegay, 1995). These factors are released by the epithelial cells, macrophages, fibroblasts and vascular endothelial cells. The process of angiogenesis begins from day two of wound healing (Grotendorst et al., 1984). Reduced oxygen tension in the wound instigates the expression of a factor called "hypoxia-inducible factor" (HIF) by the vascular endothelial cells (Gerber et al., 1997). The HIF further joins with specific DNA sequences that modulate the activity of VEGF thereby inducing neovascularization. As new blood vessels reach the area of healing, the oxygen tension reverts to an average level. The oxygen available at the wound then binds to HIF and prevents its action thereby resulting in decreased expression of VEGF (Diegelmann and Evans, 2004).

1.2.2.4. Protein synthesis and contraction of the wound

Protein synthesis and contraction of the wound are the stages that prevail four to five days following injury. The amount of wound matrix set down throughout this healing stage, influences the scar strength significantly (Bullard et al., 1999).
1.2.2.4.1. Protein Synthesis

The collagen constitutes 50% of the proteins in a scar tissue, and its integration is indispensable for the process of healing (Nimni, 1974). The fibroblasts synthesize collagen and other proteins required during the healing process. The synthesis of collagen is caused by the expression of TGF-β, PDGF, and EGF (Ignatz and Massaugue, 1986). Synthesis of collagen is also influenced by the wound characteristics and factors such as age, tension, pressure, and stress in an individual (Caterson and Lowther, 1978). Maximum synthesis of collagen is observed for two to four weeks post wounding which slows down subsequently. Aberrations in healing are mainly due to irregularities in the deposition of collagen, irrespective of the primary reasons. Conditions like diabetes show weakened inflammatory cell activity along with paucities in the other healing aspects that result in reduced deposition of collagen and poor wound healing (Fahey et al., 1991). On the contrary, the formation of keloid occurs due to increased synthesis of collagen, the prevention of which remains to be investigated (Ketchum et al., 1966).

The wound matrix in the initial stages is mainly composed of fibrin and fibronectin. With the acceleration of the process of protein synthesis, the matrix of the wound alters its nature. The collagen and proteoglycans progressively substitute fibrin as constituents of the ECM (Reed et al., 1993).

Among the 23 different varieties of collagen that are recognized, the scar tissue of the skin is predominated by type I collagen fibers (Prockop and Kivirikko, 1995). After the collagen transcription and processing, the mRNA binds to the polyribosomes on the endoplasmic reticulum (ER) wherein the new collagen chains are formed. At this
point, the hydroxylation of the residues of proline and lysine occurs (Peterkofsky, 1991). The molecules of collagen form the characteristic structure of triple helix, and the nascent chains endure additional alteration by the process of glycosylation (Blumenkrantz et al., 1984). A molecule of procollagen is then released into the ECM wherein it is further processed (Prockop et al., 1998). Hydroxyproline present in the collagen is significant because it is responsible for the stable helical configuration of the latter (Zanaboni et al., 2000). The collagen which is fully hydroxylated presents higher melting temperature. The hydroxyproline when absent, i.e., the collagen synthesized under the conditions of anaerobic or Vitamin C-deficiency (scurvy), exhibits a different structure and can readily undergo denaturation at a reduced temperature (Peterkofsky, 1991).

The collagen which is led into the ECM then experiences additional changes by the breakdown of the N and C-terminal peptides of procollagen. Lysyl oxidase, an essential enzyme present in the ECM, influences the collagen to produce cross-links that are stable. The collagen on maturation shows increased intramolecular and intermolecular cross-linking. This increased cross-linking provides strength and stability to the collagen (Hornstra et al., 2003). The dermal collagen present in the normal skin is strong, greatly organized and may even approach the tensile strength of steel. On the contrary, the fibers produced in a healing tissue are smaller and randomly arranged. Therefore, the scar tissue becomes weaker and easily breaks when compared to the healthy skin. The tensile strength regained by a healed tissue does not approach the natural state. The tensile strength attained by a scar tissue can only be up to a maximum of about 80% compared to that of the normal tissue. (Diegelmann and Evans, 2004).
Another component of the dermal matrix is the elastin, responsible for the skin elasticity. Elastin is not produced and is therefore not observed in a healing wound. The absence of elastin is in charge of the increased stiffness and decreased elasticity of the scar tissue (Monaco and Lawrence, 2003).

1.2.2.4.2. Wound Contraction

Contraction of the wound initiates four to five days after wounding and lasts for two weeks approximately. This process gets prolonged in the wounds that continue to remain open at the end of two weeks. The contraction of a wound is evident in an excised type. It is because the wound edges are drawn nearer. In an incision wound, the contraction is less obvious as it only leads to shortening of the scar. Although the contraction rate differs in different anatomic sites, however, it approximately presents an average of 0.6-0.7 mm/day. The degree of contraction is usually prophesied by the amount of laxity shown by the skin at the site of the injury. A scalp wound contracts slowly compared to a wound in the buttock. The shape of the wound also affects the contraction rate. The square wounds are observed to contract more rapidly compared to circular wounds. (Monaco and Lawrence, 2003).

Contraction of the wound is cell-mediated and do not involve the synthesis of collagen. TGF-β and other growth factors are mainly responsible for this process (Clark et al., 1995).

1.2.2.4.3. Myofibroblasts in Wound healing

Contraction of the wound is enabled by the cells called myofibroblasts that are present in abundance at the periphery of the wound. Myofibroblasts are the fibroblasts that are
modified and are considered to be vital in the wound contraction (Gabbiani et al., 1971).

Myofibroblasts are composed of a cytoplasm with actin-rich microfilaments, a multi-lobulated nucleus, and an abundant rough ER. The duration for which the myofibroblasts are observed in the wound does not entirely resemble the period of contraction of the wound, although it is similar. Myofibroblasts usually appear four to six days after wounding and are perceived in the wound during the subsequent two to three weeks. After rendering their function, they are suspected of disappearing via apoptosis. It is believed that these cells strive as a ‘‘motor’’ that narrows a wound (Gabbiani et al., 1971). However, further research on collagen lattices has proposed that the fibroblasts available at the center of the wound may have a greater influence on the contraction (Ehrlich, 1988).

Thus, it can be stated that both fibroblasts and myofibroblasts play a vital role in augmenting the process of wound contraction by producing traction and contractile forces, respectively. These cells, however, show dual effects in the process of wound healing. An appropriate amount of generation of strength and deposition of the matrix is advantageous for healing while excessive production of the force and increased deposition of the matrix may result in the scarring and malfunctioning of the repaired tissues as observed in keloids and hypertrophic scars (Diegelmann and Evans, 2004).

Therefore, a clear understanding of how the forces are generated in these cells and a precise knowledge of the amount of force they produce may be valuable in the improvement of the treatment strategies for clinically challenging wounds (Li and Wang, 2011).
1.2.2.5. Remodeling

Remodeling of the scar tissue begins to preponderate roughly after 21 days of wounding. The frequency of synthesis of collagen reduces and coincides with the incidence of the breakdown of collagen. The downregulation of the synthesis of collagen is facilitated by the factors like g-interferon (Granstein et al., 1987), TNF-α (Buck et al., 1996), and the availability of the matrix of collagen (Madden and Peacock, 1968). MMPs are also associated with the collagen breakdown that occurs actively in the process of remodeling (Monaco and Lawrence, 2003).

The scar remodeling changes the nature of the wound matrix. An immature scar comprises of a disordered mass of thin fibers of collagen, which is replaced eventually by thicker fibers that are arranged parallel to the skin stresses. The number of intra and inter cross-links in the molecules also increase. As the matrix of collagen alters in nature, it turns less cellular due to the apoptosis of the cells engaged in the repair. The type I to type III ratio of the collagen fibers change and the number of proteoglycans and water (H₂O) molecules also reduce. The healthy tissue presents a basket like interlaced form that is never completely reproduced in a remodeled scar.

The process of remodeling is not as complicated as other features of wound healing. It is, however, responsible for the development of a strongly healed tissue. Remodeling is tightly linked with a noteworthy increase in the breaking strength of the wound. The power of the wound after one week of injury is only about 3% compared to the normal dermis. When the phase of remodeling begins to dominate, i.e., after three weeks, the wound bears about 20% of the strength of the normal dermis. After three months, the wound regains 80% of the power, with a substantial rise in the breaking
strength due to further remodeling. The process of remodeling although continues normally for a year after the wound is created, the scars formed, however, do not recoup the total strength of the normal dermis (Monaco and Lawrence, 2003).

To summarize, the cascade of wound healing begins with hemostasis and deposition of fibrin, which is followed by the inflammatory cell activity, predominated by the neutrophils, macrophages, and lymphocytes (Mast, 1992). The proliferation of the fibroblasts and deposition of collagen soon continues resulting in the tissue remodeling by the crosslinking of the collagen and maturation of the scar (Figure 1.4 & 1.5).

**Figure 1.4:** The Wound Healing Cascade (Monaco and Lawrence, 2003)
If any part of this sequence of wound healing gets altered, pathologic responses are created resulting in chronic wounds or fibrosis (Diegelmann and Evans, 2004).

1.2.3. Types of Wound Healing

1.2.3.1. Wound Healing by Primary Intention

The primary intention wound repair occurs by the process of re-epithelialization. In this case, the healing takes place in the wounds that involve the epidermis and only a partial damage to the dermis. The edges of the wound when approximated by sutures (stitches), staples, or adhesive tapes also heal by this way. Fixed lacerations and majority of the surgical wounds are also examples for the wounds that heal by primary intention. Primary intention can reduce scar formation. The effectiveness of primary closure is influenced by the wound size and shape. Only smaller injuries that are elliptical shaped provide better results on primary closure (You and Han, 2014)
1.2.3.2. Wound Healing by Secondary Intention

The secondary intention wound repair ensues by the formation of granulation tissue (fibrosis), wound contraction, and epithelialization. Secondary intention wounds require adequate care to avoid contamination and enable the development of granulation tissue. Untreated full skin thickness open wounds heal by this type. Secondary intention healing normally leads to noticeable and undesirable scars (You and Han, 2014).

1.2.3.3. Wound Healing by Tertiary Intention

In tertiary intention, the injuries are kept open initially and shut after many days (i.e., four to five days). It is done by suturing the wound edges together or by using skin grafts or flaps. The injured area is cleaned, debrided, and carefully observed for the initial four-five days. This type of treatment is followed for infected wounds. By the fourth or fifth day, the infected mass undergoes phagocytosis thereby leading the wound towards the proliferative stage. The wound is then closed surgically (You and Han, 2014).

1.2.4. Roles of ROS in Wound Repair

Oxygen (O₂) plays a major role in the wound healing process. It is involved in slaying the bacteria by oxidation, synthesis of collagen, angiogenesis, and epithelialization. The wound healing is therefore compromised in the conditions of hypoxia (Sen, 2009; Schreml et al., 2010). Although the presence of oxygen is essential, however, its exact role in the process of wound regeneration is not defined. Generation of energy by oxidative phosphorylation employs oxygen and releases ROS that produces oxidative injury. During the normal metabolic process, the aerobic cells continually produce
ROS which is further increased in the conditions of pathology. Although ROS supports various physiological processes, they can also pose life-threatening impairment (Kurahashi and Fujii, 2015).

The physiological and molecular influence of ROS on wound repair have been previously investigated (Sen and Roy, 2008; Schäfer and Werner, 2008; Wagener et al., 2013). The pathological influence of ROS on the inflammatory phase is defined in particular. In the phase of inflammation, the neutrophils and macrophages that reach the site of injury begin to release vast quantities of ROS in addition to proinflammatory cytokines (Goldman, 2004) and MMP (Gill and Parks, 2008). The NADPH oxidase (NOX2) activated during phagocytosis and expressed in the plasma membranes of inflammatory cells at high levels lead to the generation of massive quantities of superoxide radical anions (Darr and Fridovich, 1994; Bedard and Krause, 2007). The ROS produced, attack the invading pathogens directly, killing them and thereby aiding in phagocytosis. However, the superoxide when produced excessively results in the damage of the surrounding tissues (Dunnill et al., 2017).

Low levels of ROS respond to stimuli and functions as cellular signals (Rhee, 2006; Marinho et al., 2014). The physiological influence of ROS on angiogenesis was also explained previously (Bretón-Romero and Lamas, 2014). Optimal levels of H$_2$O$_2$ enhances the expression of the VEGF, hastening the process of angiogenesis (Sen et al., 2002; Roy, 2006).

ROS also aids in the process of epithelialization by eliciting the expression of EGF and KGF. It is brought about by H$_2$O$_2$ (Marchese et al., 2003; Goldkorn et al., 1998).
H$_2$O$_2$ also induces the fibroblasts to generate TGF-α (Vivekananda et al., 1994). It thus favors the epidermal cell migration and proliferation (Kurahashi and Fujii, 2015) (Figure 1.6).

Although low levels of ROS have a positive influence on wound healing, however, increased production of ROS results in oxidative stress that can hinder the healing process (Dunnill et al., 2017). Increased and persistent levels of ROS have been observed in vivo and have been found to be closely related to the poor healing of chronic wounds (Schäfer and Werner, 2008). At the molecular level, in addition to ROS-mediated transcription that causes continued secretion of pro-inflammatory cytokines and generation of MMPs, excessive ROS can also alter or reduce ECM proteins directly and indirectly (via activation of proteolysis). It also results in the impaired functioning of the dermal fibroblast and keratinocytes (Moseley et al., 2004).
Excessive ROS can also be detrimental to angiogenesis. Increased ROS affects the signaling complexes by producing an imbalanced redox homeostasis, leading to poor wound repair. Aging, immunodeficiency, malnutrition, and diabetes are considered as the classic reasons for poor wound repair. Under these circumstances, increased oxidative damage is detected (Dunnill et al., 2017).

Thus it can be stated that the low levels of ROS are crucial in efficient wound repair (Rodriguez et al., 2008), whereas excessive levels of ROS leads to cell damage and poor wound healing (Ponugoti et al., 2013).

The maintenance of the normal levels of ROS is vested on a specialized protein group called antioxidants. The antioxidants aim at eliminating the harmful effects of ROS.

1.2.4.1 Antioxidative Enzymes and ROS

The intracellular levels of ROS are influenced by its generation or elimination by the antioxidant system. Living cells comprise of numerous antioxidants that avoid or mend the injury instigated by ROS, and also control the redox-sensitive signaling pathways. The major anti-oxidative enzymes that are considered vital in all living cells are catalase (CAT), superoxide dismutase (SOD), glutathione (GSH)-related glutathione s-transferase (GST), glutathione peroxidase (GPx) and peroxiredoxin (PRDX) etc. (Dunnett et al., 2017; Kurahashi and Fujii, 2015).

Regulation of ROS by Antioxidants: The superoxide formed in the tissue is oxidized and reduced to H_{2}O_{2} and O_{2} by SOD or a spontaneous reaction. H_{2}O_{2} is further detoxified by CAT, GPx, and PRDX to evade the Fenton reaction. This reaction is
toxic and happens in the presence of the transition metal ions such as iron or copper and produces hydroxyl radicals, i.e., the most damaging ROS. The CAT along with the peroxidases convert H$_2$O$_2$ into H$_2$O while the CAT alone also neutralizes H$_2$O$_2$ to O$_2$ and H$_2$O. The net result is the efficient conversion of the two possibly harmful species, i.e., superoxide and H$_2$O$_2$, into H$_2$O (Weydert and Cullen, 2010) (Figure 1.7).

![Figure 1.7: Role of Antioxidants in the process of Wound Healing](Kurahashi and Fujii, 2015)

Thus the antioxidants greatly influence the process of wound repair. Various studies conducted using genetically altered animals and pathological models have exposed the benefits of these enzymes in the process of healing (Kurahashi and Fujii, 2015). However, the level understanding is still limited. Further studies are therefore essential to explain the particular role of the antioxidants in regulating the ROS in the process of wound repair.
1.2.5. Abnormal Wound Healing

1.2.5.1. Fibrosis: Keloids and Hypertrophic Scars

The typical structural components of the tissue being replaced by damaged, non-functional and excessively accumulated scar tissue are defined as fibrosis. Excessive scar formation is considered to be the most important biological marker that indicates fibrosis. Various medical conditions are related to the increased formation of the scar tissue. E.g., keloids and hypertrophic scars (Kovacs, 1991; Diegelmann and Evans, 2004).

The scar formed during wound healing is associated with collagen III and abundance in myofibroblast activity and number (Volk et al., 2011). Reduced scar formation due to the myofibroblast senescence is a planned response to wound repair that limits fibro-genesis. The ECM controls this process by the expression of CCN1/CYR61 which acts through integrin-mediated oxidative stress induction (Jun and Lau, 2010).

Increased density of mast cells is also indicative of fibrosis (Gruber, 2003; LeRoy et al., 1991). Mast cells encompass specialized enzymes that can process procollagen. It is also stated that under the influence of these cells, abnormal peptides are generated that can further result in the synthesis of collagen thereby leading to fibrosis (Kofford et al., 1997).

Keloids are classic examples for fibrosis and serve as biochemical and cellular markers (Rahban and Garner, 2002). The fibroblasts isolated from keloids have been observed to produce about two to three times increased collagen compared to those separated from the healthy skin tissues (Diegelmann et al., 1979). Keloids show an increased expression of TGF-β and a upregulation of TGF-β receptors (Babu et al.,
1992; Chin et al., 2001). Another gene, Fussel-15 has also been associated with the migration of fibroblasts into the wound site and formation of keloids. (Arndt et al., 2011).

Hypertrophic scars are yet another class of fibrosis that is also represented by excessive scar formation and collagen accumulation (Ehrlich et al., 1994).

1.2.6. Delayed/ Impaired Wound Healing- Chronic Wounds
Chronic wounds fail to follow the normal stages of healing in a systematic and well-timed way. Usually, the chronic wounds are arrested at the inflammatory phase. The etiology varies. However, these wounds share a set of features in common, such as the amplified activity of pro-inflammatory cytokines, ROS, proteases, and increase in the number of senescent cells. Continuous infection and stem cells deficiency/dysfunction are also characteristic of chronic wounds (Frykberg and Banks, 2015) (Figure 1.8).

**Figure 1.8:** Normal versus Chronic Wound Healing (Demidova-Rice et al., 2012)
Chronic wounds are grouped into the following types: pressure ulcer, vascular ulcer (arterial and venous), and the diabetic foot ulcer (Rice et al., 2012). Some of the common characteristics shared by all these ulcers include a prolonged phase of inflammation (Eming et al., 2007), persistent contaminations (Edwards and Harding, 2004), and generation of drug-resistant microbial biofilms (Wolcott et al., 2008). The epidermal and dermal cells also fail to respond to the reparative stimuli in this condition (Rice et al., 2012). All these features prevent the wounds from healing and render them chronic. Diabetic foot ulcers are the most common among these chronic wounds.

Diabetic foot ulcer is a serious condition resulting out of diabetes mellitus (DM), a metabolic disorder. It is observed in 15% of the individuals who have DM (Brem and Tomic-Canic, 2007), and leads in 84% of all factors responsible for diabetic related amputations of the lower-leg (Turns, 2013).

1.2.7. Diabetes and Wound Healing

DM is a progressive and chronic endocrine disorder that mainly leads to hyperglycemia (increased blood glucose levels). In a global platform, due to an increase in the prevalence, diabetes is stated to be one among the major health problems (Alam et al., 2014).

A survey conducted worldwide testified that DM affects nearly 10% of the population per year. The number of people affected by diabetes are on the rise and are influenced by factors such as aging, population explosion, urbanization and increasing prevalence of physical inactivity and obesity (David et al., 1997). According to a recent estimate, India will see the greatest increase in the number of individuals with
diabetes and the total number is believed to rise from 31.7 million as recorded in the year 2000 to 79.4 million in the year 2030. In a global scenario, the total number of people with diabetes is expected to increase from 171 million as recorded in the year 2000 to 366 million in the year 2030. Thus the worldwide occurrence of DM is estimated to upsurge from 2.8% in 2000 to 4.4% in 2030. Therefore, the human population appears to be in the midst of an epidemic of diabetes all over the world (Wild et al., 2004).

The International Diabetes Federation (IDF) has presented a comprehensive statistic report that, for every 10 seconds, two individuals will develop diabetes, and another two will die of conditions related to diabetes (International Diabetes Federation, 2007). Therefore, diabetes poses a serious issue related to public health that leads to a socioeconomic problem in several countries.

According to WHO, DM is a heterogeneous metabolic disorder that presents with the features of chronic hyperglycemia with disturbances in the metabolisms of carbohydrate, protein, and fat. Chronic hyperglycemia results in impaired function or failure of various organs such as eyes, kidneys, heart, etc. and structures such as the nerves, and blood vessels. DM is a leading cause of illness and death all over the world. Type 2 DM is the most common cause. The increased incidence of obesity and reduced levels of healthy lifestyle practices are held responsible for this condition. DM is expected to continue as a major health problem owing to its serious complication, especially end-stage renal disease, gangrene of the lower extremities and blindness in adults. Increased evidence suggests that diabetes show an increase in the oxidative stress. This increase is due to the amplified production of ROS and
decreased antioxidant support, a process that begins at an early stage and deteriorates as the disease progresses (Anusha et al., 2007).

1.2.7.1. Diabetic Wounds
Diabetic wounds are slower to heal and are invariably associated with recurrent infections. Diabetic patients usually suffer from complications of the lower extremity such as peripheral neuropathy, vascular problems, and ulcerations that result in diabetic foot infections. Nearly 25% of diabetic patients are prone to developing foot complications that may last a lifetime (Singh et al., 2005). Foot ulceration is the most common complication of diabetes with 25 to 80% of an estimated annual incidence (Lavery et al., 2007).

1.2.7.1.1. Diabetic Foot Ulcers
These ulcers occur due to neuropathic damage of the musculoskeletal balance as well as a compromise in immunity due to dysfunction of the leukocyte and peripheral vascular diseases that further complicate these wounds with infection (Marston, 2006).

Most of the foot ulcers if left untreated transforms into diabetic gangrene. This finally results in the amputation of the lower limb as observed in nearly 80% of the cases (Pecoraro et al., 1990; Larsson et al., 1998; Agardh et al., 1998; Aljadi and Yusoff, 2003; Khanolkar et al., 2008; Frykberg and Veves, 1996; Mayfield et al., 2003; American Diabetes Association, 1999). More than 50% of the diabetic wounds can increase the risk of amputations below-knee exponentially (Armstrong and Lipsky, 2004; Lavery et al., 2006; Lipsky et al., 2004), which pointedly increases the mortality rates. It also leads to a reduced quality of life with massive social,
psychological, and economic penalties (Zgonis et al., 2008; Tentolouris et al., 2004; Schofield et al., 2006; Butoille et al., 2008). Majority of the diabetic foot ulcerations involve the toes (Reiber et al., 1998). If the ulcers are not treated within the stipulated time, the amputation of the affected limb becomes inevitable (Robbins et al., 2008).

1.2.7.2. Factors responsible for impaired healing in Diabetes

Altered wound healing in DM is influenced by many factors (Cunha, 2000; Caputo et al., 1994; Goodson and Hunt, 1979; Boulton and Vileikyte, 2000; Economides and Aristidis, 2000; Kamal et al., 2006). Some of the factors are related to a diabetic patient’s tendency to develop diseases such as atherosclerosis or renal failure. The factors may also be related to the chances of developing neuropathy. The decreased capacity to deal with infection is another factor that contributes to impaired healing. Various cellular, metabolic, and biochemical factors are also responsible for the altered healing in DM (Greenhalgh, 2003).

Macrovascular and Microvascular Diseases: DM leads to both macro and microvascular diseases. Atherosclerosis finds an increased incidence in case of diabetes. Thus, impairment in the wound healing is common due to vascular stenosis/occlusion. Atherosclerosis can lead to the formation of emboli in the proximal vessels that usually result in the damage of toes. The decrease in the blood flow leads to the inadequate supply of oxygen that renders healing difficult and impossible. Therefore, it is imperative to check for insufficient oxygen supply to the wound as a prime cause of poor healing.
Microvascular disease is yet another cause of impaired healing in diabetes. It may result in the formation of a thickened perivascular basement membrane at the capillary level. The after-effect of this thickening is unclear. It may, however, alter the micronutrient distribution. Studies have also suggested that the vascular permeability may also increase (Shimomura and Spiro, 1987).

**Edema:** Edema also hampers wound healing in diabetes. The basement membrane thickening may pose a difficulty to the migration of the leukocytes. This deposition of the ECM may ‘‘trap’’ the inflammatory cells and may add to prolonged infection (Greenhalgh, 2003).

**Uremia:** Uremia also affects wound healing. Individuals with DM are predisposed to renal diseases. Augmented loss of protein in the urine as observed in diabetes predisposes the individual to edema, which, further, leads to poor wound repair (Yue et al., 1987).

**Neuropathy:** The increased tendency to develop neuropathy is considered as the main contributor to the development of impaired wounds in DM. People with DM are inclined to develop neuropathy in the lower limbs. The neuropathy involves all the type of nerves. The loss of protective reflexes is the major consequence of neuropathy. The diabetic patients tend to walk on the wounds being unaware that they exist and also do not feel any pain. In the absence of the sensory input, the pain felt due to the pressure is ignored which eventually leads to the ischemic death of the area involved.
The development of plantar ulcers over the metatarsal heads in the diabetic patients is also attributed to neuropathy. In addition to a sensory deficit, diabetic neuropathy also leads to the damage of the motor and sympathetic nerves. The muscles that maintain the arch of the foot lose the feedback to their normal functioning. Therefore, the arch may be lost in the diabetic individuals resulting in increased pressure on the heads of the metatarsals. The lack of sensation along with increased pressure results in pressure necrosis that is most commonly observed in the head of the second metatarsal. The protective callus that is formed also surge the chances of necrosis.

The loss of sympathetic supply may also lead to a foot that fails to sweat. It will result in skin cracking due to the absence of adequate moisture and further upturns the risk of developing wounds (Greenhalgh, 2003).

**Impaired Resistance to Infection:** Another critical issue with DM is the increased possibility of developing an infection. There are multifactorial reasons for the weakened resistance to infection. The cracking of the skin provides an increased area and environment for the bacteria to grow. The bacterial flora may also be changed by the impaired sweating. Impaired glucose tolerance may also increase the risk for infection. Conditions of hyperglycemia may provide a nutrient-rich medium for the bacterial growth, and thus cause impairment of the local defenses. A hyperglycemic environment also hampers the functions of the leukocytes (Greenhalgh, 2003).

**Decreased Production of Growth Factors/ Destruction of Growth Factors:** Animal experiments have shown that there are specific cellular vagaries that result in poor wound healing in DM. In the 1990s, a considerable amount of research focused on the possible use of recombinant growth factors in hastening wounds healing in
diabetic animals. The application of growth factors to the diabetic wounds showed beneficial effects on healing (Brown et al., 1994; Greenhalgh et al., 1993; Greenhalgh et al., 1990). The problems encountered during the healing of chronic wounds and how growth factors worked were also studied.

One theory stated that diabetic wounds failed to produce adequate growth factors (Greenhalgh, 1996). Decreased production of the growth factors reduce the stimuli for the wound to heal normally. The observation that the application of growth factors was valid also further supported the growth factor deficiency theory. The investigators with the help of molecular detection techniques have explained that there is a reduction in the expression of numerous cytokines, such as insulin-like growth factor (IGF-I, IGF-II), KGF, etc. These observations were made in the wounds of diabetic animals in comparison to control (Brown et al., 1997; Frank et al., 1995; Werner et al., 1994).

Another proposition was that the diabetic wounds show a greater destruction of growth factors. This assumption was reinforced by studies in chronic pressure ulcers wherein MMPs were observed in increased levels in the fluid of the chronic wound in comparison to acute wound (Trengove et al., 1999).

Similar findings were also made in the diabetic wounds of animals. Neely et al. found that numerous MMPs were augmented in the diabetic wounds in comparison non-diabetic controls. These observations were made in the genetically diabetic mouse model (Neely et al., 2000). It, therefore, appears, that both reduced production and increased destruction of the growth factors are equally accountable for the poor healing in DM.
Hyperglycemia: Impaired glucose tolerance also leads to altered tissue repair in diabetic wounds (Goodson and Hunt, 1979). On the contrary, the prevention of hyperglycemia improves healing in diabetic animals (Goodson and Hunt, 1979). A clear understanding of the relationship between the altered glucose control and altered healing would further aid in the development of new strategies for healing the impaired tissue.

1.2.8. Wound Care

Wound care has found more advances in the past two decades than the yesteryears. This advancement is due to an increased explanation made available at the molecular level for the process of wound healing. The expanding scientific knowledge synchronized with the technological advancement has delivered significantly improved methods of wound-care that can heal wounds with minor complications (Monaco and Lawrence, 2003)

Gathering first-hand information about the unique biological markers and mechanisms related to the normal and pathologic responses of wound healing, aids in the development of new treatment strategies to manage these clinical conditions. Procuring the core knowledge of wound repair will also enable the wound care specialists to hone their treating skills and improve the responses of wound repair.

Different kinds of synthetic drugs are available to enhance the wound healing process. However, most of them focus only on preventing inflammation and growth of microbes at the site of the injury. The exogenous growth factors delivered at the wound site would mimic the natural microenvironment of tissue formation and repair
is assumed to be effective therapeutically. However, these procedures are expensive and are therefore inaccessible to the general population.

Regardless of finding new methods for promoting the process of wound healing, wound care has found solace in the roots of medicine and is embracing some of the traditional therapies used ages ago.

1.2.9. Traditional Medicines in Wound Healing

Traditional medicine encompasses the knowledge that has been amassed over many generations. The traditional medicines were in use much before the advent of modern medicinal practices. It mainly includes Ayurveda, Siddha, Unani, Homeopathy, and Naturopathy. Various other methods such as the Iranian, Islamic, Vietnamese, Chinese, Acupuncture, Muti, etc. are also included under traditional medicines and have evolved from all over the globe. It may even comprise the use of folk medicine, i.e., ancient therapies practiced and circulated by laypersons.

The World Health Organization (WHO) has stated a clear definition for traditional medicines. According to WHO, the traditional medicines are ‘the healthcare practices, methods, knowledge, and beliefs including plant, animal, and mineral-based medications, spiritual therapies, manual techniques, and exercises. These modalities are implemented singularly or in combination to diagnose, treat, and prevent sicknesses and maintain the well-being’ (WHO Report, 2008). Nearly 80% of the inhabitants in certain Asian and African countries depend on the use of traditional medicines to meet their basic health-care needs even today. The traditional medicine when espoused outside its culture, is popularly termed alternative/ complementary medicine. (Dorai, 2012).
The terms ‘complementary/alternative medicine’ is often used interchangeably with traditional medicine. Alternative medicine is a healing method that does not come under the purview of the conventional medicine. It is influenced by various historical and cultural traditions, and lack appropriate scientific validation. They include a wide set of practices of health-care. These practices are however not assimilated into the dominant health-care system (Dorai, 2012).

Traditional systems of medicine in India such as Ayurveda, Unani, Homeopathy, and folk medicine have been continually working on improving the process of wound healing. They have asserted that the medications employed are not only anti-inflammatory and antimicrobial but also has regenerating and rejuvenating effects (Datta et al., 2011).

Honey and Ghee have constantly been used in Ayurveda for wound healing. Use of Honey in the treatment of wounds, both acute and chronic is also practiced and approved in modern medicine.

Further, the plants and their extracts also have been identified to possess enormous ability in the wound management and treatment. The phytomedicines used for wound healing are not only inexpensive and affordable but are also supposedly safe as hypersensitive reactions are seldom encountered with their use (Raina et al., 2008). Although some of these plants have been scientifically screened for their wound healing potential in diverse pharmacological models and patients, however, the ability of most of them remains unknown. The active chemical constituents have also been identified in a few cases (Biswas and Mukherjee, 2003). Some of the medicinal plants known for their wound healing abilities are listed below (Table 1.1).
Table 1.1. Medicinal plants having wound healing activity (Rawat et al., 2012)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Plant</th>
<th>Family</th>
<th>Part Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Acacia catechu</em> Willd.</td>
<td>Mimosoideae</td>
<td>Bark</td>
</tr>
<tr>
<td>2</td>
<td><em>Acalypha indica</em></td>
<td>Euphorbiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>3</td>
<td><em>Achyranthes aspera</em></td>
<td>Amaranthaceae</td>
<td>Latex</td>
</tr>
<tr>
<td>4</td>
<td><em>Adhatoda vasica</em> Linn.</td>
<td>Acanthaceae</td>
<td>Leaves, stem</td>
</tr>
<tr>
<td>5</td>
<td><em>Adhatoda zeylanica</em> M.</td>
<td>Acanthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>6</td>
<td><em>Agrimonia pilosa</em></td>
<td>Rosaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>7</td>
<td><em>Aloe vera</em></td>
<td>Liliaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>8</td>
<td><em>Alstonia scholaris</em></td>
<td>Apocynaceae</td>
<td>Latex</td>
</tr>
<tr>
<td>9</td>
<td><em>Alternanthera brasiliana</em> Kuntz.</td>
<td>Amaranthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>10</td>
<td><em>Anacardium occidentale</em> L.</td>
<td>Anacardiaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>11</td>
<td><em>Areca catechu</em> L.</td>
<td>Arecaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>12</td>
<td><em>Argemone mexicana</em> L.</td>
<td>Papaveraceae</td>
<td>Latex</td>
</tr>
<tr>
<td>13</td>
<td><em>Aristida setacea</em> Retz.</td>
<td>Poaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>14</td>
<td><em>Arnebia densiflora</em> Ledeib</td>
<td>Boraginaceae</td>
<td>Root</td>
</tr>
<tr>
<td>15</td>
<td><em>Barleria prionitis</em> L.</td>
<td>Acanthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>16</td>
<td><em>Begonia fallox</em> DC.</td>
<td>Begoniaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>17</td>
<td><em>Betula alnoides</em> B.H.</td>
<td>Betulaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>18</td>
<td><em>Blepharis maderaspatensis</em></td>
<td>Acanthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>19</td>
<td><em>Boschniakia himalaica</em></td>
<td>Orobancheaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>20</td>
<td><em>Brassica juncea</em> L.</td>
<td>Brassicaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>21</td>
<td><em>Buxus wallichiana</em></td>
<td>Buxaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>22</td>
<td><em>Calendula officinalis</em> L.</td>
<td>Asteraceae</td>
<td>Flower</td>
</tr>
<tr>
<td>No.</td>
<td>Species Name</td>
<td>Family</td>
<td>Parts</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td>23</td>
<td><em>Callicarpa arborea</em> Roxb.</td>
<td>Verbenaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>24</td>
<td><em>Calophyllum inophyllum</em></td>
<td>Clusiaceae</td>
<td>Leaves, Bark</td>
</tr>
<tr>
<td>25</td>
<td><em>Calotropis gigantea</em> L.</td>
<td>Asclepiadaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>26</td>
<td><em>Calotropis procera</em> Br</td>
<td>Asclepiadaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>27</td>
<td><em>Cassia alata</em> L.</td>
<td>Fabaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>28</td>
<td><em>Cassia auriculata</em> L.</td>
<td>Fabaceae</td>
<td>Leaves, Bark</td>
</tr>
<tr>
<td>29</td>
<td><em>Catharanthus roseus</em></td>
<td>Apocynaceae</td>
<td>Flower</td>
</tr>
<tr>
<td>30</td>
<td><em>Chasalia curviflora</em> Wall.</td>
<td>Rubiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>31</td>
<td><em>Chenopodium album</em> Linn.</td>
<td>Chenopodiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>32</td>
<td><em>Cirsium sinense</em> C.B.Clarke.</td>
<td>Asteraceae</td>
<td>Root</td>
</tr>
<tr>
<td>33</td>
<td><em>Cirsium veratum</em> Spreng.</td>
<td>Asteraceae</td>
<td>Root</td>
</tr>
<tr>
<td>34</td>
<td><em>Cissampelos pareira</em> L.</td>
<td>Menispermaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>35</td>
<td><em>Cleome viscosa</em> L.</td>
<td>Cleomaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>36</td>
<td><em>Combretum flagrocarpum</em> C.B.Clarke.</td>
<td>Combretaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>37</td>
<td><em>Commiphora mukul</em> Engl.</td>
<td>Burseraceae</td>
<td>Bark</td>
</tr>
<tr>
<td>38</td>
<td><em>Commelina benghalensis</em></td>
<td>Commelinaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>39</td>
<td><em>Curcuma domestica</em> V.</td>
<td>Zingiberaceae</td>
<td>Tubers</td>
</tr>
<tr>
<td>40</td>
<td><em>Curcuma longa</em> L.</td>
<td>Zingiberaceae</td>
<td>Rhizomes</td>
</tr>
<tr>
<td>41</td>
<td><em>Cyanotis villosa</em> Spreng.</td>
<td>Commelinaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>42</td>
<td><em>Datura stramonium</em> L.</td>
<td>Solanaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>43</td>
<td><em>Daucus carota</em> L.</td>
<td>Apiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>44</td>
<td><em>Dendrophthoe falcate</em> L.f.</td>
<td>Loranthaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>45</td>
<td><em>Diotacanthus albiflorus</em> Benth.</td>
<td>Acanthaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>46</td>
<td><em>Dodonaea viscosa</em> Linn.</td>
<td>Sapindaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>Page</td>
<td>Species</td>
<td>Family</td>
<td>Part</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------</td>
<td>--------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>47</td>
<td><em>Eupatorium odoratum</em> L.</td>
<td>Asteraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>48</td>
<td><em>Euphorbia antiquorum</em> L.</td>
<td>Euphorbiaceae</td>
<td>Stem</td>
</tr>
<tr>
<td>50</td>
<td><em>Euphorbia hirta</em> L.</td>
<td>Euphorbiaceae</td>
<td>Latex</td>
</tr>
<tr>
<td>51</td>
<td><em>Euphorbia pilosa</em></td>
<td>Euphorbiaceae</td>
<td>Latex</td>
</tr>
<tr>
<td>52</td>
<td><em>Ficus bengalensis</em> L.</td>
<td>Moraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>53</td>
<td><em>Ficus religiosa</em> L.</td>
<td>Moraceae</td>
<td>Bark</td>
</tr>
<tr>
<td>54</td>
<td><em>Gelsemium elegans</em></td>
<td>Loganiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>55</td>
<td><em>Glycyrrhiza glabra</em></td>
<td>Fabaceae</td>
<td>Root</td>
</tr>
<tr>
<td>56</td>
<td><em>Gymnema sylvestre</em> R.Br.</td>
<td>Asclepiadaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>57</td>
<td><em>Hibiscus rosa sinensis</em></td>
<td>Malvaceae</td>
<td>Leaves, root</td>
</tr>
<tr>
<td>58</td>
<td><em>Hippophae rhamnoides</em> L.</td>
<td>Elaeagnaceae</td>
<td>Leaves, fruit</td>
</tr>
<tr>
<td>59</td>
<td><em>Ixora coccinia</em> L.</td>
<td>Rubiaceae</td>
<td>Flowers</td>
</tr>
<tr>
<td>60</td>
<td><em>Jatropha curcas</em> L.</td>
<td>Euphorbiaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>61</td>
<td><em>Jatropha gossypifolia</em> L.</td>
<td>Euphorbiaceae</td>
<td>Resin</td>
</tr>
<tr>
<td>62</td>
<td><em>Lawsonia inermis</em> Linn.</td>
<td>Lythraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>63</td>
<td><em>Lepidium sativum</em> Linn.</td>
<td>Cruciferae</td>
<td>Leaves</td>
</tr>
<tr>
<td>64</td>
<td><em>Lycopodium serratum</em></td>
<td>Lycopodiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>65</td>
<td><em>Melastoma malabathricum</em></td>
<td>Malastomataceae</td>
<td>Bark</td>
</tr>
<tr>
<td>66</td>
<td><em>Mentha viridis</em> L.</td>
<td>Lamiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>67</td>
<td><em>Mikania micrantha</em></td>
<td>Asteraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>68</td>
<td><em>Morinda citrifolia</em></td>
<td>Rubiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>69</td>
<td><em>Morinda pubescens</em></td>
<td>Rubiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>70</td>
<td><em>Moringa oleifera</em> Linn.</td>
<td>Moringaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>71</td>
<td><em>Murraya paniculata</em> Linn.</td>
<td>Rutaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>No.</td>
<td>Botanical Name</td>
<td>Family</td>
<td>Part</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>72</td>
<td><em>Musa sapientum</em></td>
<td>Musaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>73</td>
<td><em>Napoleonaea imperialis</em></td>
<td>Lecythidaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>74</td>
<td><em>Nerium indicum</em> Mill</td>
<td>Apocynaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>75</td>
<td><em>Ocimum sanctum</em> Linn.</td>
<td>Labiatae</td>
<td>Leaves</td>
</tr>
<tr>
<td>76</td>
<td><em>Ophiorrhiza mungos</em> L.</td>
<td>Rubiaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>77</td>
<td><em>Pinus roxburghii</em></td>
<td>Pinaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>78</td>
<td><em>Ploygonatum officinale</em> A.</td>
<td>Liliaceae</td>
<td>Root</td>
</tr>
<tr>
<td>79</td>
<td><em>Pongamia pinnata</em> Vent.</td>
<td>Fabaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>80</td>
<td><em>Pothos scandens</em> L.</td>
<td>Araceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>81</td>
<td><em>Psychotria flavida</em></td>
<td>Rubiaceae</td>
<td>Root</td>
</tr>
<tr>
<td>82</td>
<td><em>Quercus infectoria</em></td>
<td>Fagaceae</td>
<td>Root</td>
</tr>
<tr>
<td>83</td>
<td><em>Raxid paeoniae</em></td>
<td>Paeonaceae</td>
<td>Root</td>
</tr>
<tr>
<td>84</td>
<td><em>Rubia cordifolia</em> Linn.</td>
<td>Rubiaceae</td>
<td>Bark, Root</td>
</tr>
<tr>
<td>85</td>
<td><em>Rubus sanctus</em></td>
<td>Rosaceae</td>
<td>Root</td>
</tr>
<tr>
<td>86</td>
<td><em>Rungia repens</em> L.</td>
<td>Acanthaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>87</td>
<td><em>Scoparia dulcis</em> L.</td>
<td>Scrophulariaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>88</td>
<td><em>Sesamum indicum</em> Linn.</td>
<td>Pedaliaceae</td>
<td>Seed</td>
</tr>
<tr>
<td>89</td>
<td><em>Sida acuta</em> Burm.F.</td>
<td>Malvaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>90</td>
<td><em>Smilax zeylanica</em> L.</td>
<td>Smilaceae</td>
<td>Rhizome</td>
</tr>
<tr>
<td>91</td>
<td><em>Solanum xanthocarpum</em> Linn.</td>
<td>Solanaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>92</td>
<td><em>Sphaeranthus indicus</em> Linn.</td>
<td>Asteraceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>93</td>
<td><em>Taxus wallichiana</em> Zucc.</td>
<td>Taxaceae</td>
<td>Bark</td>
</tr>
<tr>
<td>94</td>
<td><em>Tectona grandis</em></td>
<td>Verabinaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td>95</td>
<td><em>Tephrosia purpurea</em> Linn.</td>
<td>Fabaceae</td>
<td>Leaves</td>
</tr>
</tbody>
</table>
The plants chosen for the present study were GG and NI. The healing benefits of GG and NI on both acute and chronic (diabetic) wounds although mentioned have not been scientifically standardized and validated. Therefore GG and NI were considered in the present study to experimentally evaluate their wound healing abilities before their practical introduction to wound care.

1.2.9.1. Honey

Honey is an amalgamation of many sugars that yield a highly viscous and sweet solution (Figure 1.9). It is procured from the nectar of flowers or other secretions from the plant and is produced by the Honeybee (Apis mellifera). The solution also includes an addition of various other enzymes originating from the bees.
Honey is a combination of fructose (40%), glucose (30%), sucrose (5%) and water (20%) approximately. It comprises of various amino acids, vitamins, minerals, antioxidants, and glucose oxidase. The glucose oxidase generates $\text{H}_2\text{O}_2$, and gluconic acid, that provides an acidic pH (3.2-4.5) to Honey (Sato and Miyata, 2000).

Honey owing to its highly viscous nature is unique in rendering a moist healing environment to the wounds (Bittmann et al., 2010). The hyperosmolarity of Honey enables the absorption of exudates from the wound and permits healing in a moist environment. Honey is also known for its antibacterial, antifungal and anti-inflammatory properties (Adams et al., 2008; Mavric et al., 2008; Kwakman et al., 2010; Molan, 2001; Molan, 2002).

Honey is being used in the management of wounds since many centuries. In addition to wounds, Honey is also employed in the treatment of burns, cataracts, skin ulcers
and diarrhea (Dunford et al., 2000; Grover and Prasad, 1985). The medicinal uses of Honey are also described in the holy books such as the Bible, the Quran and the Torah (Lusby et al., 2002; Namias, 2003). The Egyptians first recognized the uses of Honey in wound healing in 2000 BC (Dunford et al., 2000). They have also employed Honey as a beauty cream and as a preservative in the process of embalming the dead. *Ayurveda* regards Honey as the ‘Nectar of Life’ and uses it extensively to treat several diseases (Grover and Prasad, 1985). Numerous studies in the initial days of the 20th century, have acknowledged the use of Honey in treating burns and have also affirmed its benefits in the treatment of wounds (Philips, 1933; Voigtlander, 1937).

### 1.2.9.1.1. Use of Honey in Ayurveda

In *Ayurveda*, Sushruta was conscious about the significance of wound healing and has pronounced sixty measures (*Shasthi Upakramas*) for wound healing (*Vrana Ropana*). The application of *Madhu* (Honey) is one among them.

“*Vaatalam guru sheetam cha raktapitakaphapaham*

*Sandhatru cchedanam ruksham kashayam madhuram madhu* ||”

Honey is sweet (*madhura rasa*). It also has an astringent added as an end taste (*Kashaya anu rasa*). It is known for its heaviness (*guru guna*), dryness (*ruksha*) and cold nature (*sheeta*). It effects the *doshas* in the following manner: It intensifies *vata*, dissolves *kapha* and stabilizes *rakta* and *pitta*. It endorses the process of healing.

The great classic of *Ayurveda*, i.e., ‘*Ashtanga Hridaya*’, describes the healing benefits of Honey as below:

“*Chakshushayam Chedi tritshleshmavishahidmaasrapittanut*

*Mehakushtakrimicchardishwaasakaasaatisaarajit* ||

*Vranashodhana sandhaanaropanam vaatalam madhu* ||”
Honey is beneficial for the eyes. It satiates thirst, scrapes *kapha*, decreases the deleterious effects, and halts hiccups. It also heals the urinary tract disorders, worm invasions, bronchial asthma, cough, nausea, diarrhea, and vomiting. Honey cleanses the wounds, heals even deeper wounds quickly, and initiates granulation tissue formation. Consumption of freshly procured Honey from the beehive helps to gain weight and is also a mild laxative. On the contrary, the older and stored Honey brings about fat metabolism and aids to lose weight. Ayurveda also glorifies another exceptional feature of Honey, i.e., “*Yogavahi*.” *Yogavahi* is a material that can penetrate the deepest of the tissues. Honey used along with other herbal medicines augments their healing properties and also aids them in reaching the deepest of the tissues (Krishna, 2005).

### 1.2.9.1.2. Use of Honey in wound healing

Various studies on animals and humans have shown the benefits of Honey in increasing wound contraction and re-epithelialization (Hejase et al., 1996; Iftikhar et al., 2010). Honey also inherits the ability to increase the granulation tissue formation and stimulating the growth of the tissues. It also reduces edema, inflammation and regulates the collagen synthesis (Bittmann et al., 2010). Honey is also capable of reducing the occurrence of postoperative adhesions in the intra-abdominal tissues (Aysan et al., 2002). Honey owns the ability to deodorize the wound and also lessen the pain (Bittmann et al., 2010).

Topical application of Honey on wound induces the early formation of healthy granulation tissue, relieves pain, and lowers the occurrence of hypertrophic scars and post-burn contracture. Honey being easily available and less expensive serves as an
idyllic dressing in the treatment of burns (Subrahmanyam, 1991). Wounds dressed in Honey showed an early reduction of inflammation, improved infection control, and faster wound repair in comparison to silver sulfadiazine treated wounds which presented with continued inflammatory reaction and loss of re-epithelialization (Subrahmanyam, 1998). Honey is shown to induce early sterility in the burn area and faster healing of burn wound when compared to the boiled potato peel (Subrahmanyam, 1996). The benefits of Honey impregnated gauze over amniotic membrane dressing in partial thickness burns were analyzed previously. In this study, the burn wounds treated with Honey healed faster with a less residual scar as compared to the amniotic membrane or polyurethane film (Subrahmanyam, 1994; 1993). The healing nature of the Honey in burn wounds is shown to be increased by the addition of antioxidant and polyethylene glycol 4000 (Subrahmanyam, 1996b) It is also shown that storing the skin grafts in Honey has advantages in treating the burn wounds (Subrahmanyam, 1993b). Recently, it has been demonstrated that no significant difference exists between the collagen dressing and conventional dressing involving Honey concerning the healing of burn and chronic wounds (Singh et al., 2011). The Honey applied topically on the non-healing wounds of the lower limb resulted in gradual normalization of the features of epithelial and connective tissues with noteworthy changes in the p63 positive epithelial cell population, recurrence of membranous E-cadherin and optimal collagen I and III depositions (Barui et al., 2011). It has also been shown that the daily application of Honey and aloe vera gel provides significant protection against Klebsiella pneumoniae B5055 infection in burn wounds (Kumari et al., 2010). Honey dressing, when compared to Silver Sulfadiazene dressing, has shown to improve the process of wound repair and render early sterilization of the wound. Honey also presented a better outcome in preventing
hypertrophic scars and post-burn contractures and reduced the need for debridement regardless of the time of application (Baghel et al., 2009). Mullai and Menon had shown that the local brand of Honey (Khadikraft Honey) has best anti-bacterial activity against *Pseudomonas aeruginosa* in comparison with an imported variety of Honey-Manuka from Australia, Heather from United Kingdom (Mullai and Menon 2007).

**1.2.9.1.3. Use of Honey – Research update**

The properties of Honey related to wound repair are influenced by factors such as species of the bee, geographical site, botanical origin, processing and conditions of storage. A variety of wounds are treated with natural and unprocessed Honey procured from different sources worldwide (Molan, 1999; Al-Waili, 2001; 2003; 2004a; 2004b; 2005). Various types of Honey such as Tualang Honey, Leptospermum Honey, Ulmo Honey and Manuka Honey have been identified to own wound healing and antibiotic properties (Khoo et al., 2010; Robson and Cooper, 2009; Sherlock et al., 2010; Tan et al., 2009).

The chief medical-grade Honey accepted for therapeutic use are Manuka and Revamil. Manuka Honey originates from New Zealand and Australia. It is manufactured from the manuka bush (*Leptospermum scoparium*) and has been extensively tested all over the world (Molan and Betts, 2004; Molan, 2006). This medical Honey has been specially processed and irradiated and shown to have antibacterial activity and helps in speedy regeneration of the tissue at the wound site (Simon et al., 2009). However, almost every sub-continent uses a particular type of Honey endemic to its area that has presented positive results in wound healing (Dorai, 2012).
Honey has been proved to be useful in treating different types of wounds such as lower limb wounds (Barui et al., 2011), laminectomy (Farrokhi et al., 2011), radiation-induced oral mucositis (Khanal et al., 2010), diabetic foot ulcers (Moghazy et al., 2010), intestinal anastomotic wounds (Ergul and Ergul, 2010), and burn wounds (Baghel et al., 2009). Honey was also effective on the wounds impaired by radiotherapy (Robson and Cooper, 2009) and many other types of wound management during oncology care (Bardy et al., 2008).

The commonness in the existence of antibiotic-resistant microbial species in the current wound scenario has led to a reconsideration of the healing benefits of traditional medications such as Honey. This review is because Honey is believed to possess antibiotic and antibacterial properties (Karayil et al., 1998). It is also testified to have a preventive influence on nearly sixty bacterial species, comprising aerobes, anaerobes, gram-positive and gram-negative bacteria (Molan, 2002). The antibacterial properties of Honey are attributed to its low water activity and presence of H$_2$O$_2$ (Wahdan, 1998). The Honey being primarily a saturated mixture of two monosaccharides, most water molecules present therein binds to the sugars. Only a few are available to the micro-organisms, thereby making the environment unfriendly for their growth. When the Honey is topically applied, the H$_2$O$_2$ gets stimulated by the process of dilution with body fluids. This H$_2$O$_2$ is gradually liberated and serves as an antiseptic. The relatively acidic pH of Honey also inhibits the growth of several bacteria (Waikato Honey Research Unit, 2006). The antibacterial activity of Honey is also affirmed by the complex interaction between its components such as H$_2$O$_2$, flavonoids, phenolic acids, methylglyoxal, bee defensin-1 and many other unidentified substances (Schepartz et al., 1966; Kwakman and Zaat, 2012; Mandal and Mandal, 2011).
The antifungal properties of Honey on certain yeast and species of *Aspergillus* and *Penicillium* and the common dermatophytes have also been identified (Molan, 2002, Wilkinson and Cavanagh, 2005). Numerous reports have published the efficacy of Honey in swiftly clearing infections from wounds and protecting them from further infections (Molan, 2002; Cooper et al., 2002; Allen et al., 1991; Willix et al., 1992; Fruncillo and Digregorio 1983). Recently it has been shown that both Honey and silver based wounds dressings have a similar influence on the viability of a cell (Tshukudu et al., 2010). Tualang Honey (a type of Malaysian Honey) is shown to have better control on the *Pseudomonas aeruginosa* infection and resulted in better wound contraction in full skin thick burn wound model (Khoo et al., 2010). The advantages of Honey as a dressing material for the wounds have been studied and compared with many other regular drugs used in dressing the wounds such as Silver sulfadiazine (Baghel et al., 2009), Mafenide acetate (Hashemi et al., 2011) and Povidone-iodine (Shukrimi et al., 2008).

Majtan and his co-workers demonstrated that Honey is also effectively involved in the activation of human keratinocytes, by up-regulating the expression of the growth factors such as IL-1β, TGF-β, TNF-α, and MMP-9. It has also been proved that Honey significantly encourages type IV collagen degradation in the skin through the activation of MMP-9 (Majtan et al., 2010). Use of Honey in wound dressing can also influence the process of angiogenesis (Rossiter et al., 2010).

### 1.2.9.2. Ghee

Ghee is a type of clarified butter that has found its origin from the Indian subcontinent. It is used in cuisines of the South Asian and Middle Eastern Countries, religious rituals and preparations of traditional medicines (Figure 1.10).
Cow’s Ghee, the butterfat procured from the cow’s milk is acclaimed to hold numerous medicinal properties such as an energy source, cooling in nature, revitalizing, confers beauty and luster; improves memory, stamina and also surges the intellect. It also encourages longevity, and serves as an aphrodisiac and safeguards the body from several diseases (Chunekar, 1960).

Cow’s Ghee is also proved to be beneficial in wound healing (Charde et al., 2006). The antibiotics present in Ghee displays powerful antimicrobial activity (Prasad et al., 2006).

### 1.2.9.2.1. Use of Ghee in Ayurveda

*Ashtanga Sangraha*, yet another classic of *Ayurveda*, describes the potential benefits of Ghee as below:

“Sahasraviryam vidhibhighrutam karmasahasrakrut ||”

Ghee is enormously powerful and possesses thousands of benefits.

“Shastam dhismrutimedhagnibalau shukrachakshusham||”
Ghee surges the digestive fire. It improves absorption and assimilation. It nourishes the elusive essence of all the tissues in the body called “Ojas,” reinforces the brain and nervous system, and enhances memory. It is responsible for the lubrication of the connective tissue. Use of Ghee renders the body flexible and, in smaller proportions, is “tri-doshic.” Ghee appeases vata and pitta and is also acceptable in moderation for kapha. Ghee should be cautiously used by individuals who already possess increased cholesterol levels or are suffering from obesity. Ghee should not be administered in conditions of high ama (toxicity) (Chunekar, 1960).

“Dhee kaanthismruthikaarakam balakaram medhokaram shuddhikrudpataagham shramanaashanam swarakaram pittapaham pushtidam||”

Cow’s Ghee exhibits numerous properties. It enhances memory and intelligence; invigorates the skin increasing its radiance, detoxifies the body, and provides energy. It stabilizes vata and pitta; increases the voice clarity; nourishes the body, and improves digestion. It is highly effective in treating the disorders of the eye and also enhances the semen quality and quantity. It serves as an Indian alchemy (Rasayana) and is the best source of healthy fat (Chunekar, 1960).

_Ghrita_, a formulation of cow’s Ghee along with herbal drugs commonly used in _Ayurveda_, displays powerful immune-stimulant, antioxidant and hepatoprotective activity (Fulzele et al., 2002). _Brahmi ghrita_, a medicinal preparation comprising of cow’s Ghee and leaves of _Brahmi_, exhibits significant memory enhancement, antidepressant and anticonvulsant activities (Achliya et al., 2005; 2004).
1.2.9.2.2. Use of Ghee in wound healing

The major components of Ghee include saturated and polyunsaturated fatty acids (PUFAs) such as the linolenic acid (n-3), linoleic acid (n-6) and oleic acid (n-9) (Bhavbhuti, 2006). The PUFA present in the Ghee is capable of controlling cell to cell interaction and intracellular signal transduction (Ruthig and Meckling-Gill, 1999). The n-3 and n-6 PUFA also enable the proliferation of epithelial cells in vitro which serves as an essential step in wound repair (Calder, 2001). The PUFAs also serve as primary precursors for several lipoic mediators, for example, the arachidonic acid which plays a major role in the inflammatory process involving vascular constriction, chemotaxis, adhesion, transmigration and cellular activation (Calder et al., 2002).

Animal experiments involving topical application of PUFA rich codfish oil showed better healing indicated by the early wound contraction. It affirms the active role and possible therapeutic benefits of fatty acids on wound repair (Cardose et al., 2004). Recently, it has been observed that a combination of Ghee and Curcuma longa had a significant role in altering the inflammatory responses and improving the parameters of repair in the process of healing when compared to the hyaluronic acid application (Habiboallah et al., 2008).

1.2.9.2.3. Use of Ghee – Research update

Ghee has been proved to have advantageous therapeutic effects on wound healing. The wound healing benefits of a polyherbal formulation in Ghee called the Hingvadya ghrita was studied on rat wound models. It revealed that the medicine when applied topically increased the tensile strength and wound contraction in incision and excision models respectively. The healing was much better compared to the application of Framycetin sulfate. The histological findings presented better re-epithelization,
keratinization, collagen rearrangement, and reduced fibrosis in the wounds treated with the Ghee based medicinal preparation (Fulzele et al., 2002). Another formulation containing 50% Ghee and 50% of 0.5% neomycin showed significant results in wound healing in comparision to the untreated groups and those treated with neomycin alone. The results were presented in the form of early wound contraction, faster wound closure, shorter period of re-epithelization, increased tensile strength, and quicker tissue regeneration at the wound site (Prasad and Dorle, 2006). Recently, the cow’s Ghee in combination with the oil of flax seeds, fruits of Phyllanthus emblica, the resin of Shorea robusta, and Yashada bhasma has also shown better healing abilities on both excision and incision wounds. Among the various combinations, the wounds treated with cow’s Ghee, the resin of Shorea robusta and Yashada bhasma exhibited comparatively improved wound closure, increased collagenation and greater tensile strength (Datta et al., 2011).

1.2.9.3. Glycyrrhiza glabra (GG)

Scientific Name: Glycyrrhiza glabra (Family: Fabaceae)

Indian/Sanskrit Name: Yashti-madhu, Yashti-madhuka, Mulhathi, Jethi-madh

Common Name: Liquorice, Licorice, Mulethi (Hindi), Atimadhura, Jeshtamadhu
(Kannada)

GG is a plant of medicinal value, grown in several parts of the world. Being an herbaceous perennial legume, GG commonly inhabits southern Europe and certain parts of Asia, such as India (Figure 1.11).
GG is an oldest and extensively used herb from the primeval times in Ayurveda. It is used as a medicine as well as a flavoring agent that mask the disagreeable taste of other medicinal preparations (Biondi et al., 2005). They have also been used by the Egyptian, Chinese, Greek, Indian, and Roman civilizations in various medicinal preparations. In the traditional medical system, the roots and rhizomes of GG have been used clinically for years owing to their anti-inflammatory, anti-ulcer, expectorant, carminative, antimicrobial and anxiolytic properties (Asl et al., 2008).

GG is known to possess potent antioxidant, free radical scavenging (Di Mambro et al., 2005) and anticonvulsant abilities (Nassiri-Asl et al., 2007). The main taproot of GG collected for medicinal use, is soft, fibrous and shows a bright yellow interior. The constituents of the root of GG include triterpenes, polycaccharides, pectin, sugars, flavonoids, amino acids, mineral salts, etc. The most important and biologically active component of the root of GG is a triterpenoid saponin (Sharma et al., 2015).
The root extracts of GG have been extensively used in Japan for more than sixty years in the treatment of conditions such as chronic hepatitis. They are also known to restrict the activities of viruses. The expectorant, antitussive, mild laxative and anti-aging properties of GG have also been reported previously (Ravikumar et al., 2013; Sadul et al., 2012).

**1.2.9.3.1. Use of GG in Ayurveda**

GG has been popularly known as Yashti-madhu in Ayurvedic pharmacy for thousands of years. It is a vital component of Ayurveda and is one among the essential drugs in Sushruta Samhita.

*Yashtimadhu* has *madhura rasa* (sweet). It also possesses the following medicinal properties: sheeta virya (cold potency) and madhura vipaka (better post digestion effect) (Singh, 2007). It is a *vata-pitta shamaka* (pacifies the aggravated vata and pitta) (Ministry of Health & Family Welfare). As mentioned by Charaka, if administered with *kshira* (cow’s milk), it would exhibit greater properties of *medhya* (intellect or cognition). Yastimadhu also exhibits excellent properties of *vrana shodhana* (wound healing) (Singh, 2007).

**1.2.9.3.2. Use of GG in wound healing**

The studies carried out using scientific parameters have ascertained the wound healing, antiulcer, anti-inflammatory and skin regeneration properties of GG (www.energy.sk/info/menu_x2055x.asp). Wound healing properties although stated; however, there is a dearth of the available literature to support the same adequately.
1.2.9.3.3. Use of GG- Research update

The root extracts of GG are sweet and are commonly used as a flavoring agent to mask the bitter taste in medicinal preparations (Chopra and Chopra, 1958). GG is also known to possess the following properties: antispasmodic, demulcent, diuretic, emollient, expectorant, and laxative. It is a moist and comforting herb that detoxifies and safeguards the liver. It is also a powerful anti-inflammatory agent, used in the treatment of arthritis and mouth ulcers (Ravikumar et al., 2013; Sadul et al., 2012). GG is known to exhibit hormonal effects that resemble the ovarian hormone. GG is an active component of the medicines administered for cough and cold and is also involved in the treatment of catarrhal inflammations of the urinary tract. It is also internally administered for the treatment of conditions such as Addison's disease, bronchitis, asthma, peptic ulcers, complaints of allergy and following steroidal therapy (Asl et al., 2008).

The favorable effects of GG can be ascribed to various mechanisms. The constituents of GG display steroid-like anti-inflammatory activity, resembling that of hydrocortisone. It is partly due to the inhibition of the enzyme vital for various inflammatory reactions, i.e., phospholipase A2 (Okimasu et al., 1983). *In vitro* studies have also documented that glycyrrhizic acid (an active component of GG) inhibits the activity of the cyclooxygenase and formation of prostaglandins (i.e., prostaglandin E2). It also indirectly inhibits the aggregation of the platelets thereby reducing the rate of inflammation (Okimasu et al., 1983; Ohuchi and Tsurufuji, 1982). Some of the constituents of GG possess substantial antioxidant properties, i.e., glycyrrhizin and glabridin. These components have been observed to inhibit the production of ROS at the inflammation site (Akamatsu et al., 1991; Wang et al., 2001). Anti-carcinogenic
properties are also exhibited by glycyrrhizin and other constituents of GG. Research has revealed that GG inhibits the proliferation of abnormal cells, as well as the formation of tumors in skin cancer. However the exact mechanisms influencing these activities are still being investigated (Nishino et al., 1984; Liu et al., 1998). The formulations of GG used in the treatment of ulcers do not suppress the release of gastric acid, unlike other anti-ulcer medicines. Rather, they encourage healing by increasing the production of mucous and blood supply to the damaged gastric mucosa, thereby resulting in better healing (Van et al., 1981; Goso et al., 1996).

1.2.9.4. Nerium indicum (NI)

Scientific Name: *Nerium indicum* Mill/ *Nerium oleander* L. / *Nerium odorum* Sol. (Family: Apocynaceae)

Common Name: Kaner, Kanagile, Indian oleander

NI is an evergreen shrub that grows to a maximum height of about five meters. It is a native of the Mediterranean region; often cultivated as an ornamental shrub in gardens worldwide. The leaves are long, green, leathery, simple, whorled/pairs of three, linear-lanceolate, and about 9-14 centimeter in length. The flowers are soft, sweet-scented, and present in the form of clusters at the end of every branch. The flowers may grow as single or double cymes with eye-catching colors such as white, pink or red (Figure 1.12).
NI is considered to be one among the most poisonous garden plants that are toxic in all its parts. Although toxic, the plant is known for its benefits in folk medicine (Chaitanya et al., 2010).

NI has many medicinal properties. It serves as an astringent, anthelmintic, aphrodisiac, acrid, stomachic, febrifuge, diuretic, emetic, expectorant, cardiotonic, anti-cancerous drug, etc. It is used in the treatment of asthma, renal and vesicle calculi, chronic stomach and skin related problems, snake bites, joint pains, leprosy, cancer, ulcers, etc. (Sandeep et al., 2009).

The leaves and flowers of NI are used in the treatment of malaria and also as a traditional medicine in the termination pregnancy. The powdered roots of NI are used as an external remedy for the treatment of hemorrhoids and ulcers around genitals.
Wound healing properties of NI have also been mentioned. The decoction of the leaves of NI was externally used for the treatment of scabies and to lessen swelling by local herbalists (http://natureconservation.in/nerium-indicum-kaner-complete-detail-updated).

1.2.9.4.1. Use of NI in Folk medicine

NI is traditionally used as a folk remedy by the herbalists in the treatment of various conditions such as dermatitis, abscesses, wound healing, eczema, psoriasis, sores, warts, corns, ringworm, scabies, herpes, skin cancer, asthma, dysmenorrhea, epilepsy, malaria, tumors, etc. They are also used as abortifacients, heart tonics, and emetics. The macerated leaves of NI have been topically applied for the treatment of conditions such as dermatitis, alopecia, superficial tumors and syphilis (Dey and Chaudhuri, 2014). NI leaf extract is also being used in the treatment of gingivitis and as nasal drops for children (Chaitanya et al., 2010; Sandeep et al., 2009).

1.2.9.4.2. Use of NI in wound healing

NI is considered be useful in healing chronic wounds, both diabetic and nondiabetic (Chaitanya et al., 2010). It could be attributed to its antibacterial, antifungal and anti-inflammatory activities. The antioxidant activities of NI have also been identified (Dey and Chaudhuri, 2014). Although the wound healing properties of these medicinal preparations find their basis in folk medicine and are still practiced in some households, however not much light is thrown on their efficacy and mechanisms of healing. An adequate scientific evidence is therefore required to support the same, and this is possible only by extensive clinical research.