CHAPTER II

REVIEW LITERATURE

ALOE VERA

1. BOTANY OF ALOE VERA

Aloes have been used as therapeutic agent, certainly since Roman times and perhaps long before (Morton, 1961;Crosswhite and Crosswhite,1984), different properties being ascribed to the inner, colorless, leaf gel and to the exudate from the outer layers.

Family Aloe vera is in Liliaceae. The scientific name is Aloe vera (Linn.) Burm. f.

Aloe vera is a short-stemmed succulent herb. The succulent leaves are crowded on the top of their stems, spreading, grayish green and glaucous; spotted when young, 20-50 cm long, 3-5 cm wide at the base, tapering gradually to the point tip,1-2.5 cm thick; having edeges spiny and bitter latex inside. Flowers borne on the upper part of a slender stalk, 50-100 cm high. Forms of the species vary in sizes of leaves and color of flowers (Grindlay and Reynolds,1986).

The epidermis of the leaves has a thick cuticle, and beneath is zone of parenchyma which obtains pericyclic cells. The latex or yellow juice contains within the pericyclic cells. The central bulk of the leaf contains the colorless mucilaginous pulp, made up of large thin-walled mucilaginous cell containing the aloe gel itself (Klein and Penneys,1988) as shown in Figure 2.1.

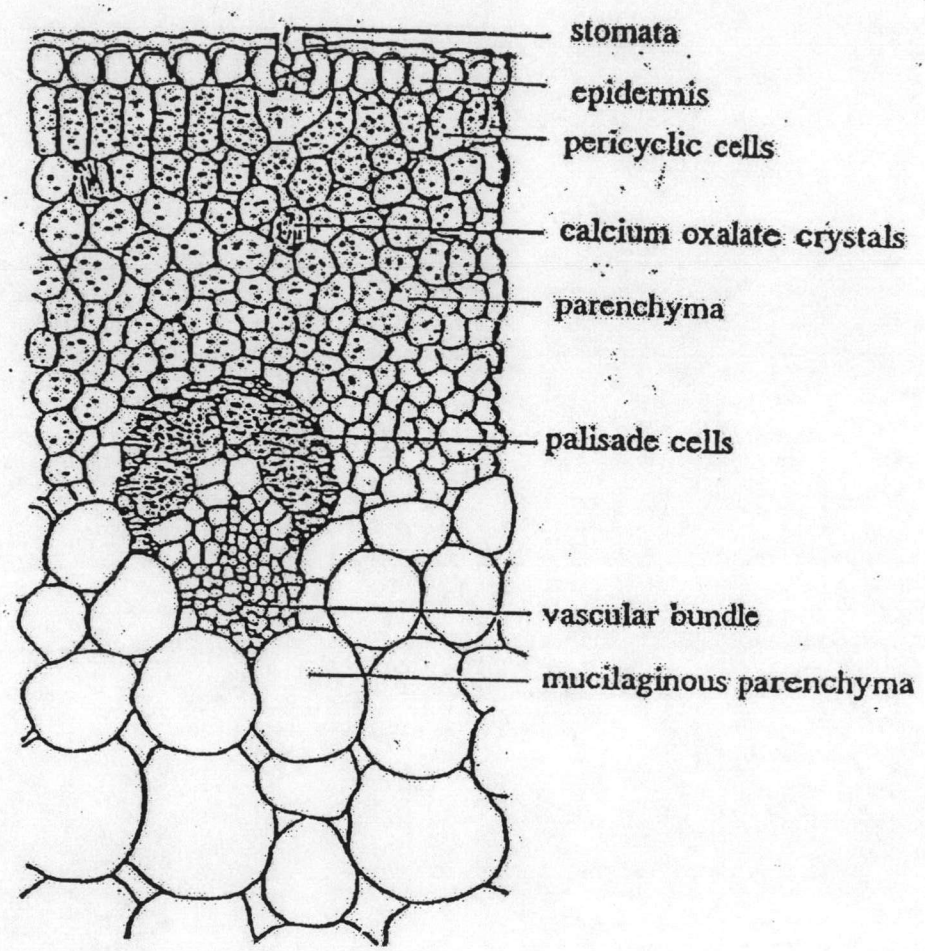

Figure 2.1 *A transverse section near the margin of the aloe leaf. (Klein et al., 1988)*

2. CHEMICAL CONSTITUENTS OF ALOE VERA

The latex portion of Aloe vera contains several anthraquinone glycosides derivatives such as aloin, barbaloin, isobarbaloin, anthranol, aloe emodin, chrysophanic acid, 1,8- dihydroxyanthraquinone (Henry, 1979; Hirata and Suga,1977 ; Robson 1982, Spoerke and Ekin,1980).

The fresh gel consist of 99.5% water and 0.5 % solid component (Gjerstad,1971;Mckeown 1983).Analysis of the solid components revealed that the largest number of active substances (97%) were in the mixed polysaccharides (Davis et al., 1981; Yagi, 1982). Besides, the solid components had been found to comprise glycoprotein, aloctin A, aloctin B (Suzuki et al., 1979); amino acids, (Waller et al.,1978), vitamins, inorganic compounds, enzymes, uric acid, salicylic acid, etc. Table 2.1 summarizes its most important compositions.

It was believed that a strong synergistic relationship existed between polysaccharides and other active substances in aloe such as amino acids and vitamins (Davis et al., 1990; Henry, 1979 ; Leung,1977,1978; Waller, 1978). Certain amino acids and vitamins showed strong anti-inflammatory activity (Hanley et al., 1982), suggesting that these substances might have a triggering effect on enzyme and polysaccharide activity needed for antiinflammation (Coats,1979). The healing properties as well as the antiinflammatory effects of Aloe vera had polysaccharides base as active ingredient, and also needed synergistic effects of their active substances (Henry, 1979; Leung, 1977, 1978; Mckeown, 1983; Waller,1978).

Table 2.1 *Chemical Compositions of Aloe vera (Vogler BK and Ernst, 1999)*

Constituent	Identification
Anthraquinones	Aloin, barbaloin, isobarbaloin, anthranol, aloetic acid ester of cinnamic acid, aloe-emodin, chrysophanic acid, resistannol
Saccharides	Cellulose, glucose, mannose, L-rhamnose, aldopentose
Vitamins	B_1,B_2,B_6 ,choline, folic acid,C, α-tocopherol, β-carotene
Nonessential amino acids	Histidine, arginine, hydroxyproline, aspartic acid, proline, glycine,alanine, tyrosine
Inorganic compounds	Calcium, sodium, chlorine, manganese, zinc, chromium, potassium sorbate, copper, magnesium,iron
Enzymes	Cyclooxygenase,oxidase, amylase,catalase,lipase, alkaline phosphatase, carboxypeptidase
Essential amino acids	Lysine, threonine,valine,leucine, isoleucine, methionine, phenylalanine
Miscellaneous	Cholesterol, triglycerides, steroids, β-sitosterol, uric acid, gibberellin, lectin-like substance, lignins, arachidonic acid, salicylic acid

PHYSIOLOGYCAL AND PHARMACOLOGICAL ACTIVITIES OF ALOE VERA

Since Biblical times the use of Aloe vera as a remedy had repeatedly come up in folklore, along with testimonials related to the healing properties of the mucilage when applied to burned or inflamed skin. The beneficial effects of aloe were so miraculous as seen more like myth than fact (Cole and Chen,1943). For the last several decades, the scientists had begun seriously probing aloe chemisty for "non- folkore".

Today, Aloe vera has been recognized as a good medicine which has properties including analgesia, anticancer, antiviral, mutagen, antiulcer, cough suppressant, hyperplycemia, antifungal, anthelmintic, antiarthritis, antiparasite, antifertility, cathartic, cosmetics, insecticide, fat production and decongestion (Norman and Bunyapraphatsara, 1992). The therapeutic effects of Aloe vera on burn wound were investigated in many studies as described later.

PHYSIOLOGY OF SKIN

The skin consists of three layers: epidermis (outmost layer), dermis (internal), and subcutaneous (innermost layer) (Figure 2.2). Within these layers of the skin are many activities.

The epidermis (outer layer):

The outermost surface of the epidermis is a dead horny layer of keratin which helps protect from the environment. Directly underneath is the stratum corneum. This layer of the epidermis helps prevent water loss and also helps protect from the harmful environment elements. This barrier helps to protect the deeper layers of the dermis, epidermis, and subcutaneous layers directly

underneath. The epidermis is the layer which is often called the 'bricks and mortar' of the skin, and where there are multiple layers of ceramides, cholesterol and free fatty acids and melanin (skin pigmentation). All providie a barrier function. It is in this layer that epidermal cells die, and where there is secretion of 'keratin' (protein) and 'sebum' (oil) which accumulate in these dead cells. The more important function in the epidermis cells is the manufacturing of lipids (moisture), fibrous proteins, and fatty acids.

The dermis (center layer):

The function of the dermis is to be able to firmly stay attached to the epidermis, and to serve as the body's regulator. It primarily consists of connective tissues-collagen and elastin. As well, the dermis is where blood vessels, nerve (heat/cold), lymphatics, hair follicles, sebaceous glands, apocrine and eccrine glands that make up the structure of the skin.

The subcutaneous (innermost layer):

The subcutaneous layer of the skin is the foundation of the skin. It is in this innermost layer of the skin that has adipose (fatty cells), arteries, veins, base of the sweat glands, and sympathetic nerves (pain). All of our skin tissue is fed from the subcutaneous layer through the blood-arteries, veins and capillaries.

The skin circulation

The circulation of the skin has unusual arrangement which accommodates several different, sometimes conficting, functional requirements: nutrition of the skin and appendages, increased blood flow to facilitate heat loss in hot conditions, and decreased blood flow to minimise heat loss in cold conditions whilst nevertheless maintaining adequate nutritional flow.

The arteries supplying the skin are located deep in the hypodermis from which they give rise to branches passing upwards to form two plexuses of anastomosing vessels. The deeper plexus lies at the junction of the hypodermis and dermis and is known as the *cutaneous plexus*; the more superficial plexus lies just beneath the dermal papillae and is known as the *subpapillary plexus*. Branches of the cutaneous plexus supply the fatty tissue of the hypodermis, the deeper aspect of the dermis and capillary networks which envelop the hair follicle and deep sebaceous glands and sweat glands. The subpapillary plexus supplies the upper aspect of the dermis and the capillary networks around the superficial appendages. The subpapillary plexus also gives rise to a capillary loop in each dermal papilla. The venous drainage of the skin is arranged into plexuses broadly corresponding to the arterial supply. Numerous shunts provide direct arteriovenous communications which play an important role in thermoregulation by controlling blood flow to the appropriate part of the dermis. The skin has a rich lymphatic drainage which forms plexuses corresponding to those of the blood vascular system (Figure 2.3).

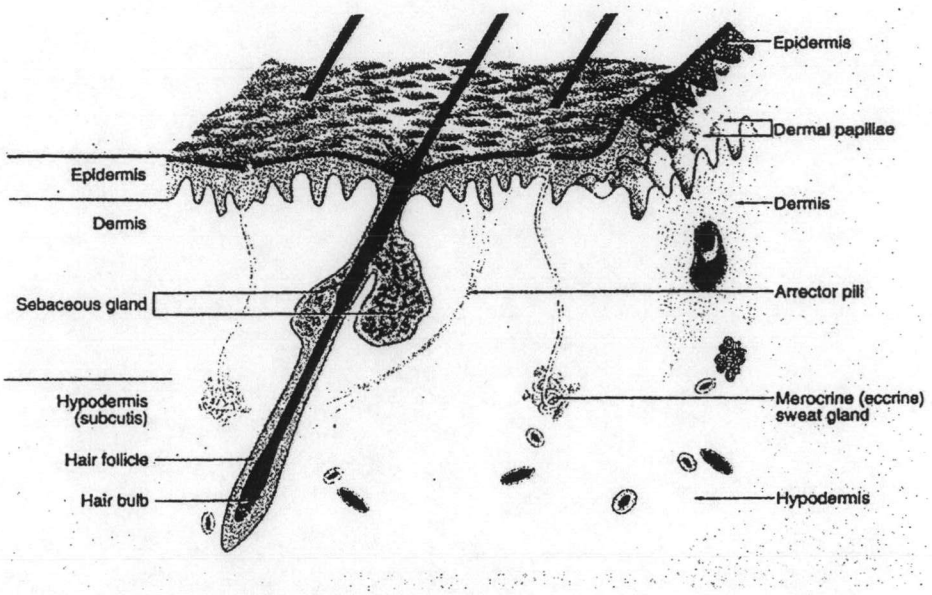

Figure 2.2 *The normal skin*

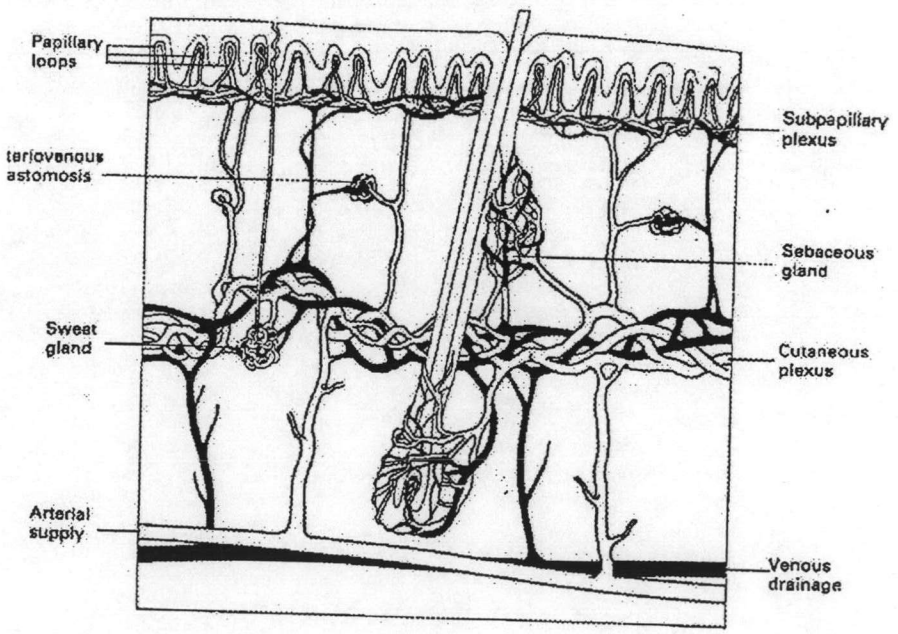

Figure 2.3 *The skin circulation*

PATHOPHYSIOLOGY OF THE BURN WOUND

1. LOCAL AND SYSTEMIC TISSUE INJURIES

The pathophysiological changes in the burn wound are characterized by effects caused by heat *per se* and superimposed on these is a pronounced acute inflammatory process. A sudden increase in body surface temperature results in prompt locally responses by the blood vessels in the area in an attempt to dissipate heat by vasodilation. A further increase in tissue temperature starts an inflammatory reaction caused by local release of inflammatory mediators and cascades of reactions then take place (Arturson, 1990).

The inflammatory response to injury, infection and antigen challenge with overproduction of chemical mediators, activation of leukocytes and endothelial cells and an alteration in circulating cytokines may all contribute to systemic effects. Thus in patients with major burns these effects are: increased susceptibility to infection, the systemic inflammatory response syndrome (SIRS) (Beal and Cerra 1994), adult respiratory distress syndrome (ARDS) (Ashbaugh et al.,1967) and multiple organ dysfunction syndrome (MODS) (Carrico et al., 1986; Bone et al., 1992), which may develop further into progressive organ failure and death. Injury elicits a response from all cells of the immune system in which cytokines from activated leukocytes can act either beneficially to provide for enhanced host resistance or deleteriously to depress the function of remote organs and cause systemic inflammation (Cioffi et al., 1993).

2. BURN WOUND CHANGES OVER TIME

Usually the burn wound initially has different depths in different regions. Often the wound is characteristically made up of several zones of tissue damage due to different heat transfer (Jackson et al., 1983). In the middle, usually the site of greatest heat transfer, irreversible skin death occurs, the *zone of coagulation*. This zone is surrounded by the *zone of stasis* characterized by a pronounced inflammatory reaction. This potentially salvageable area could be converted to full destruction by infection or drying of the wound. Outermost is the *zone of hyperemia*, which is the site of minimal cell involvement and early spontaneous recovery.

A number of distinctive phases over time postburn, mainly in the zone of stasis, can be discerned:

1. A period of rapid *local edema formation* with a maximum at about 1-3 hours postburn due to vasodilatation, increased extravascular osmotic activity (Arturson et al., 1964) and increased microvascular permeability (Arturson, 1961; Nozaki et al., 1979) A rapid degradation of hyaluronate and collagen fibers may be the reason for the increased extravascular osmotic activity behind the dramatic early drop in the interstitial fluid hydrostatic pressure. The initial suction of fluid out into the interstitium due to this so-called imbibition pressure (Lund et al., 1989) is then further accentuated by fluid leakage due to increased microvascular permeability.
2. These changes are followed by heterogeneous reductions in perfusion, the so-called *no reflow phenomenon* leading to *local tissue ischemia* and further necrosis (Zawacki, 1974). The microcirculation is compromised to the worst extent at around 12-24 hours postburn. During this period of time attempts to improve the

microcirculation by pharmacological treatment have to some extent been successful.

3. A period of transformation favouring *adhesion* on the free surfaces of *endothelial cells, platelets and leukocytes* (Von Andrin et al., 1991). This leads to leukocyte margination followed by extravasation and their migration to the injured parenchymal cells and microorganisms. Platelets removed from the circulation contribute at different levels to hemostasis and local thrombosis.
4. A later phase of *wound repair* with high rates of wound perfusion to support wound metabolic requirements and maintain adequate defence against invasive burn wound infections (Jackson et al., 1983).
5. *Burn wound microbial colonization and infection*. The burn surface is generally considered to be initially free of major microbial contamination. Gram-positive bacteria in the depths of hair follicle and sweat glands may, however, survive the heat of the initial injury (Luttherman et al.1986; Mooney et al., 1989).These bacteria may heavily colonize the wound within the first 48 hours postburn, especially if topical chemotherapy is not applied. Coincident with improvements in survival, changes in the epidemiology of infection have occurred (Mozingo et al., 1994; Pruitt et al. 1992). The microorganisms present on the wounds of hospitalized patients change with time after injury. Usually gram-positive organisms (*Staph. Aureus, Strep. Pyogenes*) during the first week postburn are superseded by gram-negative organisms (*Pseud. aeruginosa, E. coli*) during the second week. Sometimes candida species (*Candida albicans, aspergillus, phycomycetes*) are detected later. Burn wound cleaning and excision may be followed by blood–stream infections (Vindenes et al. 1993).

Burn severity is measured by the percentage of body surface burned and the depth of the injury. Depth refers to how many layers of skin are damaged. It may take 24-48 hours to completely determine the depth of the burn. The depth and percentage of body burn determine treatment.

DEPTH OF BURN

1. First degree burns:

A first-degree burn involves only the top layer of skin, or the epidermis. It may also be called a superficial burn. It is painful and red and may thin walled blisters. It will heal itself in 3-7 days and should not scar. One example of a first degree burns is a sunburn.

2. Second degree burn:

The second-degree burn or partial thickness burn involves the epidermis (top layer of skin) as well as part of the dermis layer. This type of burn is very painful. It will have large thick-walled blisters that increase in size over time. The wound may also be moist or have peeling skin. Hair remains intact. Scarring may occur. This burn may take 2-4 weeks to heal. In some cases, a skin graft may be needed to speed up the healing process and improve the appearance. Treatment includes 1-2 times a day cleansing of the wounds, removal of dead loose tissue and the use of antibiotic cream.

3. Third degree burn:

A third degree burn or full thickness burn involves the epidermis and all of the dermis. It may also involve the fat and muscle layer. This type of wound is usually not painful since the nerve endings are destroyed. The wound surface may look white , gray or brown and will have a leathery texture. This leathery tissue is called eschar. Eschar must be removed and

replaced with healthy tissue by doing a skin graft. Scarring will always occur, but may be less with treatment over time. The depth of burn are shown in Figure 2.4.

Progressive dermal ischemia resulting in progressive cell injury and death was classical phenomenon of the burn wound for 24 to 48 hours following the thermal insult (Jackson, 1953). Leukocyte sticking to vessel walls, agglutination of red blood cells, and liberation of vasoactive and necrotizing substances occurred following burn injury.

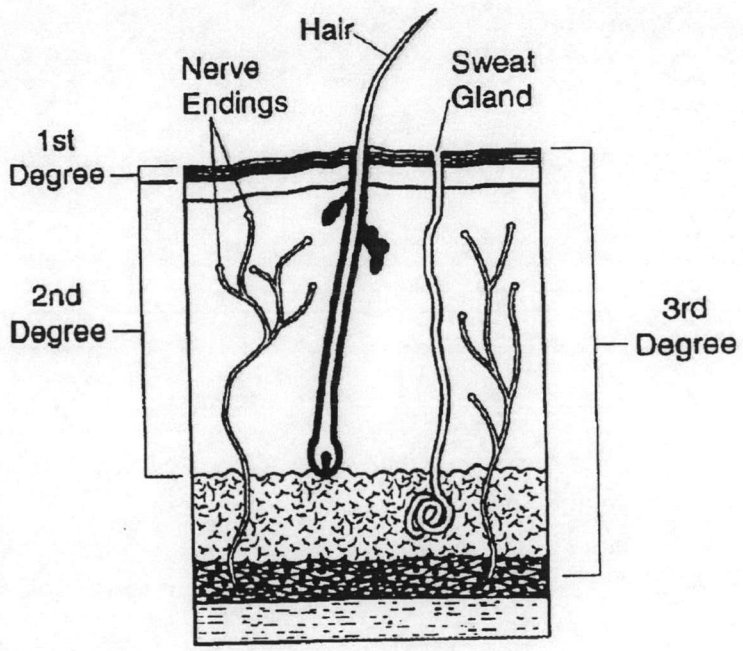

Figure 2.4 *Depth of burn*

The damaged tissue undergoes vascular sludging, thrombosis, progressive dermal ischemia, and death. Decreasing the progressive dermal ischemia could theoretically limit the amount of skin necrosis to the zone of

coagulation (Robson et al., 1980). Progressive dermal ischemia had been observed in other models as well (Heggers et al., 1993) such as frostbite injury (McCauley et al., 1993; Miller et al., 1995), electrical injury (Robson et al., 1984), and intra-arterial drug abuse (Zachary et al., 1987). These models showed that the mediators of progressive tissue damage were thromboxanes.

3. THE INFLAMMATION REACTION FOLLOWING THERMAL INJURY

Inflammation is a tissue reaction by the body to injury and typically follows burns or other skin insults. It is classically characterized by swelling (tumor), pain(dolor), redness (rubor) and heat (color) as well as loss of function (Macpherson,1992). It is thus a complex process and investigations into the therapeutic properties of the aloe gel should take account of its effects on these various symptoms. In addition, the gel may have more than one active constituent, which may be addressing different parts of the healing process. Failure to take all this into account may be responsible for ambiguities which may have arisen in the past about the efficacy of the gel. Although inflammatory processes are a natural response to injury and may hinder healing, it may also be undesirable to suppress them in an unstructured way before their purpose is accomplished. Leukocytes accompanied by fluid accumulate in the damaged tissues producing the swelling, these movements being the result of increased capillary permeability. Pain is a complex reaction following the release of short peptides and prostaglandins. The redness and heat are caused by vasodilatation which reduces blood pressure and increases circulation, although this gradually slows. Inflammation can be either caused, or intensified by invasion with micro-organism. As well as in wounds, inflammation is involved in conditions such as arthritis. Continuing research into inflammation has shown that it is a complex process involving many

biochemical pathways and a variety of agents and mediators (summarized in Davis et al., 1989). In particular these authors distinguish three components,

1. Vasoactive substances; these agents cause dilation of blood vessels and opening of junctions between cells of the ultimate capillaries, produced by altering contractile elements in endothelial cells. These factors include vasoactive amines, bradykinin and also prostaglandins.
2. Chemotactic factors; these agents increase cell motility, especially of white blood cells (leukocytes) into stressed areas. These include several proteins and peptides.
3. Degradative enzymes; these are hydrolytic enzymes which can break down tissue components. Proteases in particular participate in inflammatory states causing chemotactic factors to be released. It was also shown that aloe gel contained both an inhibitory system and a stimulatory system that influenced both inflammatory and immune responses (Davis et al.,1991).

Major inflammatory mediators, which control blood supply and microvascular permeability are shown in Table 2.2.

Table 2.2 *Major inflammatory mediators, which control blood supply and vascular permeability or modulate cell movement. The main sources are given ('Origin' column) (Arturson G, 1996).*

Mediator	Origin	Actions
Bradykinin	Kinin system (kininogen)	Vasodilatation Increased microvascular permeability Smooth muscle contraction Pain
Fibrinopeptides Fibrin split products	Coagulation system	Increased microvascular permeability PMNL and macrophage chemotaxis
C3a	Complement C3	Mast cell degranulation Smooth muscle contraction
C5a	Complement C5	Mast cell degranulation PMNL activation PMNL and macrophage chemotaxis Smooth muscle contraction Increased microvascular permeability
Substance P	Sensory nerve ending	Vasodilation Increased microvascular permeability
Histamine	Mast cells Basophils	Increased microvascular permeability Smooth muscle contraction Chemokinesis
5-Hydroxy-tryptamine (5HT=serotonin)	Platelets Mast cells	Increased microvascular permeability Smooth muscle contraction
Platelet activating factor(PAF)	PMNL Macrophages Basophils	Increased microvascular permeability Smooth muscle contraction PMNL activation
PGE_2	Cyclooxygenase pathway	Vasodilation
$PGF_{2\alpha}$	Cyclooxygenase pathway	Vasoconstriction
LTB_4	Lipoxygenase pathway	PMNL chemotaxis
LTD_4	Lipoxygenase pathway	Increased microvascular permeability Smooth muscle contraction

4. LEUKOCYTE - ENDOTHELIAL ADHERENCE FOLLOWING THERMAL INJURY

Burn injury initially produced an area of irreversible tissue destruction surrounded by a marginal zone of injury with reduced blood flow. In the postburn period ongoing inflammation and microvascular injury in the zone of stasis result in extension of the area of tissue loss.

Leukocytes, particularly polymorphonuclear neutrophils (PMNs), are central mediators of microvascular endothelial injury in many acute pathologic processes (Boykin et al., 1980, Harlan 1987, Movat 1987,Weiss 1989). PMNs have been identified as possibly contributing to the microvascular occlusion seen following burn injury both systemically and locally. Deich et al. have demonstrated an increase in PMN activation when they are exposed to burn blister fluid in vitro (Deitch et al., 1990). Nelson et al. have demonstrated an increase in the surface expression of CR3 (CD11b/CD18) on circulating PMNs following burn injury (Nelson et al.,1986). PMN-mediated injury is dependent in part on PMN adherence to the vascular endothelial cell (EC) surface and PMN-PMN aggregation in the microvasculature. PMN-EC adherence results in the formation of a microenvironment between the PMN and the EC (Harlan 1987). In this microenvironment, PMN-derived proteases and toxic oxygen products produced by both the EC and PMN exist in high locally concentrations. These highly reactive substances, partially protected from inactivation by circulating plasma anti-proteases and free radical scavengers, then produce endothelial injury resulting in intercellular gap formation, increases in microvascular permeability, edema, and thrombosis. PMN-PMN aggregation further compromises the microvascular circulation by pluging of

capillaries and post-capillary venules, extending the zone of ischemia, and subsequent tissue loss.

The sequence of events that allows the traveling of leukocytes to site host defense is designated the multistep paradigms of leukocyte recruitment. Involves important events encompassing the transvascular movement of leukocytes: 1) margination and capturing of free-following leukocytes, 2) leukocyte rolling, 3) activation and firm adhesion, 4) spreading transendothelial diapedesis and chemotactic migration of the leukocytes as shown in Figure 2.5. Different mechanisms appear to mediate leukocyte rolling and adhesion, the former is dependent on selectins expressed on endothelium (P-selectin) and leukocytes (L-selectins), whereas the latter is dependent on the integrins (CD11/CD18) found on leukocytes and their ligands (ICAM-1, VCAM-1) on endothelial cell (Yang et al., 1996).

5. INFLAMMATORY CYTOKINES

Cytokine is a term derived from Greek roots meaning "to set cells in motion". Cytokines are intercellular signaling peptides (usually between 8 and 30 kDa in mass) that can act at any range (autocrine, paracrine, endocrine). These include peptides released from microbially-stimulated leukocytes that act on other leukocyte targets. Eighteen cytokines have been given names alluding to this definition (i.e. interleukin (IL)-1 through IL-18). This terminology supplanted earlier descriptive terminology such as "endogenous pyrogen" and "lymphocyte-activating factor" on the realization that each of

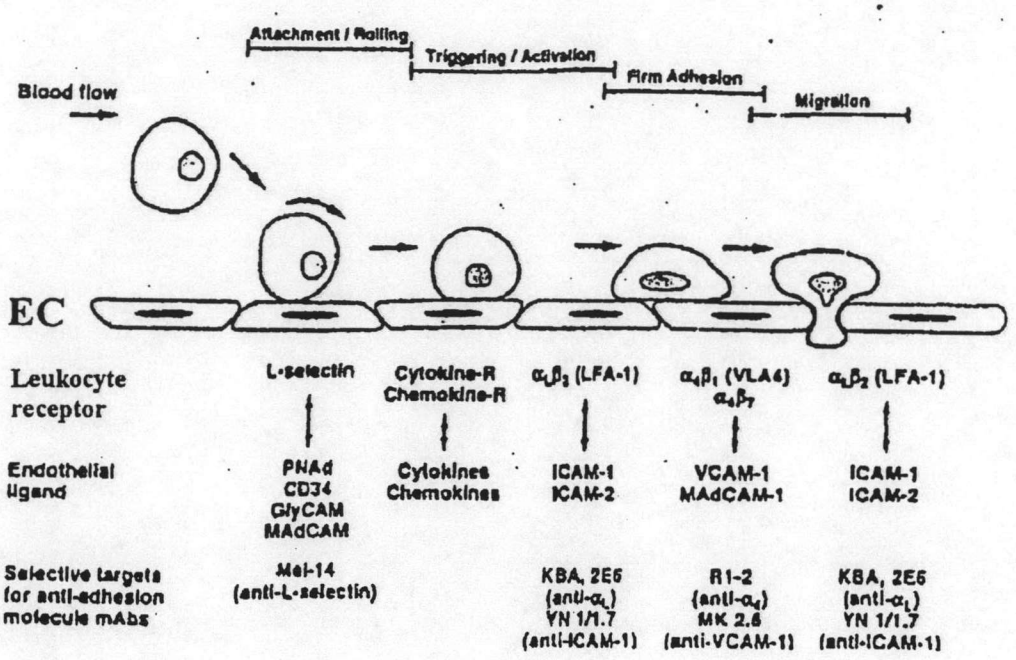

Figure 2.5 *Sequential model of leukocyte-endothelial adhesion. Extravasation of leukocytes from blood into the tissue mediated by cascade of adhesive interactions between leukocytes and endothelial cells (Yang X-D et al., 1996).*

these molecules induced a variety of biological activities that rendered activity-based names insufficient [some cytokines, such as TNF-α and transforming growth factor-β (TGF-β) have retained historical names that no longer adequately convey their biological scope]. However, the term interleukin is also overly restrictive since many cytokines given this name are produced by nonleukocyte sources and act on nonleukocyte targets. Likewise, new biological activities have been discovered for the growth factors and interferons that were originally named in the 1950s for their actions in somatic development and interference with viral replication, respectively. All

of these proteins fit under the general term "cytokine", a family with over 80 members and still growing.

Net cytokine activity in any clinical or biological context is a complex issue because of the variety and multiple activities of cytokines. Furthermore, one cytokine can radically alter (even reverse) the activity of another cytokine on a target cell. As a result, it is sometimes useful to consider cytokines in functional groups. For example, IL-2, granulocyte-macrophage colony stimulating factor, and interferon-γ promote cytotoxicity, whereas IL-4 and -13 promote antibody-mediated immunity.

In particular interest to physiologist are the so-called "inflammatory" cytokines, which include IL-1(both α and β isoforms) and TNF-α. Locally, these cytokines stimulate leukocyte proliferation, cytotoxicity, release of proteolytic enzymes, and synthesis of prostaglandins and initiate a cascade of "secondary" cytokine synthesis and secretion. One of these secondary cytokines, IL-6 is often called an inflammatory cytokine because of its temporal association with the processes just mentioned. However, many actions of IL-6 (including downregulation of IL-1 and TNF-α synthesis) are counterinflammatory, acting to keep potentially destructive inflammatory responses from overshooting (Xing et al. 1998). Systemically, these cytokines raise the thermoregulatory set point (causing fever) and, via differential influences on the expression of iron binding proteins, mediate a redistribution of iron from extracellular to intracellular sites. These alterations establish an internal environment in the host that inhibits the growth of certain bacteria. These cytokines also orchestrate a metabolic "wartime economy" that 1) reduces any energy consumption non directed at repelling the microbial invader, 2) redirects host resources to the defense effort, and 3) sets up a civil defense network that protects the "civillian" (nonleukocyte) cells from

collateral damage by antimicrobial effectors. To achieve these goals, energy consumption is reduced via direct actions of these cytokines on the central nervous system that reduce locomotor activity and increase slow wave sleep. The contractile proteins of skeletal muscle are broken down, liberating amino acids that become incorporated into specialized ("acute phase") plasma proteins synthesized by the liver. Acute phase proteins include protease inhibitors and antioxidants that neutralize the proteolytic enzymes and reactive oxygen species released by leukocytes that stray away from the site of infection and cause harm to the host's own tissues. Alterations in hypothalamic function (fever, locomotion, sleep), hepatic function (iron ,acute phase proteins), and skeletal muscle (catabolism) are all examples of cytokine-regulated adjustments to the internal environment that help the host to cope with and repel pathogenic microbial invaders.

The pathophysiological events following thermal injury are not limited to the surface effects of heat but are also related to an acute inflammatory reaction (Arturson 1996). The acute inflammatory response is largely modulated by a number of cytokines that are induced shortly after the thermal injury. The rapid overproduction of some cytokines, leukocyte activation and activation of endothelial cells leads to production of inflammatory active substances, resulting in systemic effects. IL-6 and TNF-α are the most important cytokines in the acute inflammatory reaction following thermal injury (Sparkes 1997, Arturson 1996).

TNF-α is mainly produced by activated macrophages. TNF-α regulates the production of some other cytokines. It also enhances endothelial adhesiveness for leukocytes and stimulates neutrophils and monocytes, promoting their adherence, phagocytosis, oxidative burst and degranulation. Since it has the most powerful effect on inflammation, TNF-α may be

considered to be the most important cytokine related to systemic inflammation and multiple organ failure following major trauma. Both TNF-α and IL-6 are suggested to be the important indicators of poor prognosis after thermal injury (Munster 1996, Sparkes 1997, Arturson 1996, Schindler et al. 1990).

IL-6 is produced by T and B cells, macrophages, and endothelial cells. It acts on most cells. In the liver it stimulates the production of acute-phase proteins (Bellomo et al., 1992). Furthermore, it induces B cells to differentiate into antibody-forming cells (Roitt et al., 1993). Increased levels of circulating IL-6 have been observed in burn patients especially in lethal sepsis after major burns (Drost et al., 1993; Guo., 1990;Schluter et al., 1991). IL-6 is also increased in burn blister fluid related to wound healing (Ono et al., 1995). It has been postulated that IL-6 suppresses IL-1 and TNF-α (Schindler et al., 1990). The roles of the cytokine network in thermal injury were shown in Figure 2.6.

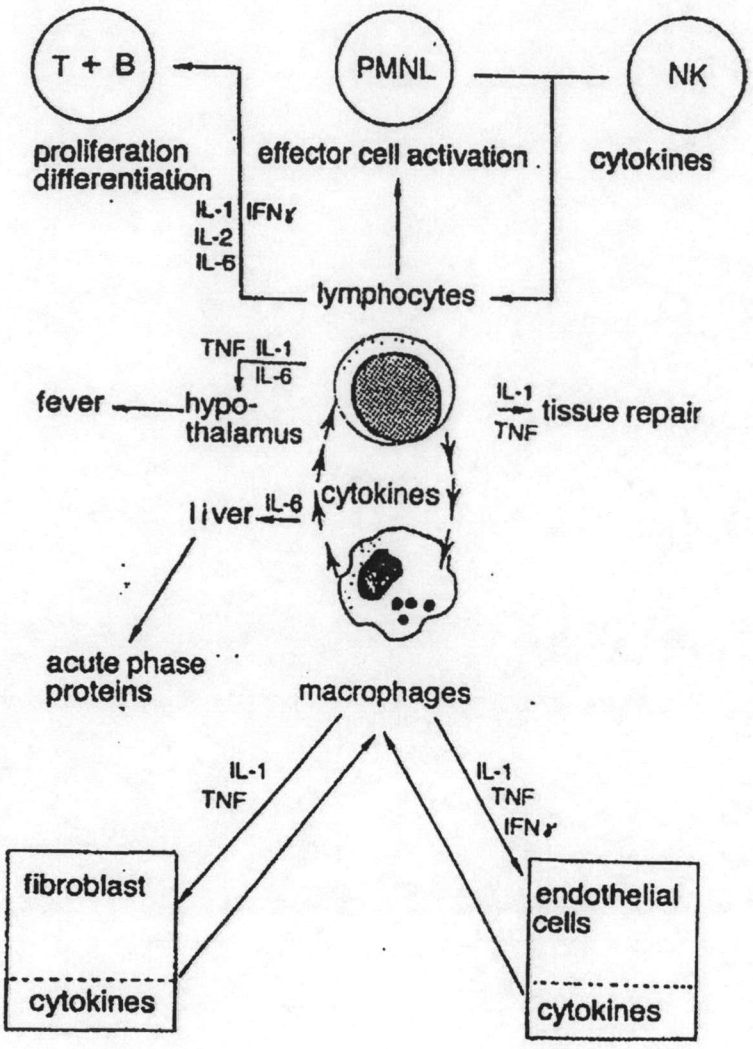

Figure 2.6 *Cytokines as communication links within the immune system, and between the immune system and other organs. Only cytokines shown to be involved in thermal injury are indicated (Arturson G, 1996).*

WOUND HEALING

Wound healing is highly complex, but orchestrated cascade of events which can roughly be divided into three overlapping phases-inflammation, granulation tissue formation and remodelling of the extracellular matrix. These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. (Raghow, 1994). Immediately after injury, there is clot formation and the earlier phases of wound repair involves inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans (PGs), which are the heterogenous, non-fibrillar components of the extracellular matrix. These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccarides, the glycosaminoglycans (GAGs). PGs and GAGs have been shown to play important roles in all the above mentioned events of wound healing (Gallo and Bernfield, 1996). For example, they prevent blood coagulation within the vascular space (Kojima et al.,1992; Parkinson et al., 1992), regulate inflammatory cell function (Forrester and Lackie, 1981; Forrester and Wilkinson, 1981) and form the major components of the ground substance (Alison, 1992), on which collagen and elastin fibers are subsequently laid. In addition, GAGs have been shown to be regulators of cellular proliferation, migration and differentiation, and of growth factor activities (Gallo and Bernfield, 1996). The synthesis of these components and their degradation, therefore, are events of great relevance in the wound healing process.

THERAPAUTIC EFFECTS OF ALOE VERA ON THE BURN WOUND

Aloe, a popular houseplant, has a long history as a multipurpose folk remedy. Aloe gel has been used for topical treatment of wounds, minor burns and skin irritations. A lot of scientific evidence for therapeutic properties of Aloe vera on the burn wound has demonstrated that the various constituents in Aloe vera may be responsible for different pharmacological actions which can be summarized as follows:

1. Antiinflammation

Inflammation is a tissue reaction by the body to injury and typically follows burns or other skin insults. It is classically characterized by swelling (tumor), pain (dolor), redness (rubor) and heat (color) as well as loss of function (Macpherson 1992). It is thus a complex process and investigations into the therapeutic properties of the gel should take account of its effects on these various symptoms.

Aloe vera was shown to be effective antiinflammatory and antiedemic agent. The carragenan was first used to induce edema formation by Winter et al. in 1962. Carragenan was colloidal extract obtained from red marine algae. It was composed of a mixture of salts of an acid sulfate of a galactose-containing polysaccharide. The hind paws of Wistar albino rats were induced edema using carragenan. The polysaccharide, mannan or 1,4-liked β-D mannopyranose, from the nondialysate of Aloe vera inhibited edema. The percentages of swelling at 1,2,3,4 and 5 hours, in treated with aloe mannan group were 22.3%, 19.1%, 20.3%, 37.1% and 48.9%, respectively, while those of untreated edema group were 24.8%, 43.5%, 63.1%, 62.9% and 72.4%, respectively (Yagi et al., 1984).

Aloe vera preparations were evaluated for topical antiinflammatory activity by the croton oil-induced edema assay. The decolorized aloe with removal of anthraquinone inhibited edema. As shown by unit of edema volume, edema in decolorized aloe (without anthraquinone)-treated group was significantly decreased when compared with untreated-edema group and diabetic edema group (Davis et al., 1988). The decolorized aloe was more effective than the colorized aloe with anthraquinone by 47% inhibition of inflammation (Davis et al., 1989).

The antiinflammatory actions of Aloe vera can be explained by the following mechanisms.

1.1 Antiprostaglandin and antithromboxane activites

As mentioned above, prostaglandins and thromboxanes , arachidonic acid metabolites, might play role in the long-term inflammatory response in tissue injuries. They had a number of different physiological effects including vasodilatation/vasoconstriction, promotion of fever and pain, and they also had an influence on the immune system. Prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) were vasodilator and platelet aggregation inhibitor. Prostglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane A_2 (TXA_2) and thromboxane B_2 (TXB_2) were vasoconstrictors and platelet aggregators (Heggers and Robson,1983, 1985).

The possible presence of prostaglandins and their effects on platelet activity in wound tissue is complex. It depends on the molecular species present and other biochemical factors in the tissue (Venton et al., 1991). Some prostaglandins are essential for normal processes in the skin such as cell

function and integrity, while others, notably thromboxane A_2 and B_2 can have devastating effects on the cells (Heggers and Robson 1983, 1985).

In 1980, Heggers et al. demonstrated high levels of protaglandin and thromboxanes in burned tissue, which have been possibly implicated in the progressive dermal ischemia seen following burning which leads to additional tissue loss. In the same year Robson et al. investigated the capability of thromboxane inhibitor i.,e., imidazole, methimazole and dipyridamole on prevention of dermal ischemia in the secondary degree burn model. Burn wounds were examined by means of immunoperoxidase technique for the presence of prostaglandins and thromboxanes. The burned tissue showed high levels of PGE_2 and TXA_2. In thromboxane inhibitor-treated animals it was shown that TXA_2 was essentially absent, while PGE_2 ,$PGF_{2\alpha}$ and PGI_2 had similar levels in untreated animals. It was suggested that thromboxane might be responsible for the progressive dermal ischemia after burning and that decreasing its production could increase dermal perfusion.

Robson et al.(1982) investigated the effects of Aloe vera on thermal burn wound in a standard guinea pig experiment. The depth of dermal ischemia was measured by perfusion with Indian ink. The result was that Aloe vera had similar effects to methylprednisolone and methimazole, giving improved perfusion of capillaries and reduction in TXB_2 and $PGF_{2\alpha}$ compared to the control animals which showed complete dermal ischemia by 24 hours. Other studies have suggested that unspecified substances in aloe gel inhibited arachidonic acid oxidation (Penneys, 1982) and thereby reduced inflammation.

Experimentally, Aloe vera was compared to a variety of antithromboxane agents (U 38450, a lodaxamide, a lazaroid). in the burn, frostbite and electrical injury. In each model, Aloe vera and therapeutic agents

were found to increase tissue survival or preserve the tissue necrosis, by actively inhibited the localized production of thromboxanes. Yet Aloe vera was found less toxic than therapeutic agents. Thus, Aloe vera not only acted as a TXA_2 inhibitor but also maintained a homeostasis within the vascular endothelium as well as the surrounding tissue (Heggers et al., 1993).

A glycoprotein component of the gel, **Aloctin A**, was shown to inhibit prostaglandin E_2 production but over a relatively long incubation time in contrast to drugs such as aspirin, (Saito et al.,1982, Ohuchi et al., 1984). It is evident that there is much complexity both the damaged tissues and plant extracts and that precise mechanisms and pathways have yet to be determined in the field of prostaglandins and their interaction with platelets.

Aloe vera provided the substance **acetylsalicylic acid** which had antiinflammatory and antiedemic activity by blocking prostaglandin and thromboxane synthesis (Davis et al., 1986).

An aqueous extract from aloe gel was also demonstrated to inhibit the production of prostaglandin E_2 from arachidonic acid in vitro and **sterols** were detected in the extract as well as **anthraglycosides** (Vazquez et al., 1996).However, a contradictory result showed the presence of cyclooxygenase enzyme in Aloe vera extracts. By having established arachidonic materials, potential precursors for the prostanoids synthesis were mixed with preincubated supernatant aloe. The presence of cyclooxygenase was demonstrated by the conversion of [^{14}C] arachidonic acid into different prostanoids including 5.3% keto-PGF_1b_α, 10.36% $PGF_{2\alpha}$, 19.23% TXA_2, 52.66% PGE_2 and 11.80% PGD_2 (Afzal et al.1991).

1.2 Antibradikinin activity

Bradikinin was both a vasodilator and potent pain producing agent at the site of acute inflammation.

Several pharmarcological studies have been performed in an attempt to identify active substance for antiinflammatory action of Aloe vera. In vivo study of Fujita and Shosuke in 1976 found that lyophilized powder aloe contained **bradykininase**. This result was confirmed by Rubel (1983) that the bradykininase activity of Aloe vera could hydrolyze bradykinin and angiotensin I to convert into angiotensin II, resulting in suppressing vasodilation and pain. The **carboxypeptidase** was reported to be enzyme in aloe vera gel that could hydrolyze bradykinin and angiotensin I in vitro (Fujita et al., 1979). The carboxypeptidase from aloe could inhibit bradykinin in vivo, and decreasing pain at the site of acute inflammation (Klein and Penneys, 1988). The other antibradykinin active material in Aloe vera which was tested on isolated guinea pig ileum in vitro, was estimated to be a **glycoprotein** (Yagi et al., 1982). It was suggested that aloe glycoproteins are the carboxypeptidase *N*-and *P*-like enzymes with proteolytic activities. The antiinflammatory action of **glycoprotein** resided in the 50% ethanol supernatant was also confirmed by Danof (1987).

1.3 Other activities

The antiinflammatory action of Aloe vera was also shown to be attributable to some active components through other activities not yet defined, in addition to those described above.

Davis et al. (1986) showed certain **amino acids, vitamins** and **RNA** in 50% ethanol supernatant aloe to have antiinflammatory activity. **Amino**

acids (Forst and David, 1979, Davis et al., 1986), **vitamins** (Davis et al., 1986, 1990) and **mannose** (Willenberg et al., 1989) in aloe could normalize the acute vascular response.

Davis et al. (1991) investigated the preparation of Aloe vera which had antiinflammatory activity. They prepared fraction Aloe vera extract with 50% ethanol, then resultant supernatant and precipitated were tested for antiinflammatory activity using the croton oil- induced ear swelling assay. It was revealed that the antiinflammatory activity resided in the supernatant of a 50% ethanol extract. Wound healing activity was found in the precipitate fraction (Davis et al., 1991, 1994). Furthermore, Davis et al. (1994) showed that the Aloe vera **sterols, lupeol, campesterol, β- sitosterol** had significantly antiinflammatory effects. Of the three sterols, lupeol caused the greatest reduction in inflammation by 37.0%. Lupeol alone reduced inflammation in dose-dependent manner. Recently Hutter et al. (1996) found a new antiinflammatory agent as cinnamoyl-C-glucosylchromone. Using croton oil-induced ear inflammmation model, at a dose of 200 μg/ear of **cinnamoyl- C- glucosylchromone** exhibited topical antiinflammatory activity equivalent to 200 μg/ear of hydrocortisone.

2. Antimicrobials: antibacterial, antiviral, antifungal

Aloe gel is bacteriostatic or bactericidal against a variety of common wound-infecting bacteria in vitro: *Staphylococcus aureus, Streptococcus pyogenes, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhosa* and *Mycobacterium tuberculosis* (Lorenzetti et al., 1964, Robson et al., 1982). **Aloe-emodin** also inhibits the growth of *Helicobacter pylori* in a dose-dependent fashion (Wang et al., 1998).

In a clinical trial, aloe gel was used to treat burns, controlled bacterial growth which was otherwise present in the untreated controls (Heck et al., 1981) and similar results were achieved in experimental trials (Rodriguez-Bigas et al., 1986, Kivett, 1989), although the relevance of casual microorganisms was challenged (Kaufman et al., 1989).

There are two useful related outcomes if antibacterial activity of aloe be confirmed. Firstly there is the obvious general antibiotic activity against pathogens exemplified in the very first paper quoted (Gottshall et al., 1949) and secondly there is activity against bacteria which may be hindering the wound healing process and contributing to inflammation (Heggers et al., 1995). A report of clinical cases suggested that the gel was bactericidal towards *Pseudomonas aeruginosa* (Cera et al., 1980). In a much later study, acemannan prevented adhesion of *P. aeruginosa* to human lung epithelial cells in monolayer culture (Azghani et al., 1995). However, some studies failed to demonstrate antibacterial activity, especially in deep wounds which became so heavily infected that death eventually ensued (Bunyapraphatsara et al., 1996). In a trial with incision wounds in rats, aloe gel was compared with standard antimicrobials and was found to speed wound healing, while the antimicrobials had an initial retardant effect (Heggers et al., 1995). It may be that antibiotic factors are released by the healing tissues in responses to aloe treatment.

Furthermore some active ingredients in Aloe vera also acted as antimicrobials in other infectious conditions. **Acemannan** acts alone and synergistically with azidothymidine (AZT) and acyclovir to block reproduction of Herpes and the AIDS virus (Kemp et al., 1990, Kahlon et al., 1991).Acemannan hydrogel (trade name is Carrisyn) is currently under investigation as a treatment for persons infected with HIV; doses are up to 250 mg qid (about one quart of raw aloe gel daily) (Duke, 1997; MacDaniel et

al., 1990). In pilot randomized controlled trials of HIV+ adults with CD4 counts, aloe did not contribute significantly to therapy with ZDV or ddI in terms of effects on CD4 counts, p24 antigen levels or viral load (Montaner et al., 1996; Singer et al., 1993).

In a randomized, controlled double blind clinical trial of 60 men suffering from an initial episode of Herpes simplex infection, those assigned to treatment with an Aloe vera extract (0.5%) in a hydrophilic cream had a significantly healing time and a higher number of healed lesions than the placebo comparison group (Syed et al., 1997).

Aloe extract treatment of guinea pig feet that had been infected with *Trichophyton mentagrophytes* resulted in a 70% growth inhibition compared with untreated animals (Kawai et al., 1998).

3. Wound healing promotion

Burn healing is regarded as a special type of wound healing and most of the skin reactions are the same. It has been pointed out however that conditions for healing would differ according to the depth of the burn and that several factors can interfere with the healing process (Kaufman et al., 1989).

A variety of studies have shown the wound healing properties of Aloe vera. Coats (1979) and Engel et al. (1981) found **amino acid, ascorbic acid, zinc, lignins, and saponins** presented in Aloe vera which increased the synthesis of collagen and counterbalanced collagen breakdown with subsequent increase in wound tensile strength. The fresh Aloe vera leaves had **lectin-like compounds** which enhanced the growth of normal human cell in tissue culture (Winter et al., 1981). Using full thickness burned wound model

maintained temperature at 250° C for 6 seconds by hot plate, the procedure was designed to compare the effect of Aloe vera extract, silvadine, salicylic acid cream and untreatment. The average time to complete healing in the untreated group was 50 days and the only significant difference was found in the Aloe vera-treated group which healed on an average time of 30 days. In addition, wound bacterial counts were effectively decreased by silvadine and by Aloe vera extract (Rodriguez-Bigas et al., 1986).

Davis et al. (1988) compared the percentage of wound reduction among varied doses of decolorized Aloe vera group, control group and diabetic group on the seventh day and fourteenth day. The diameters of the inflicted wound were measured with vernier caliper. The results in each decolorized Aloe vera groups showed significantly increased rate of healing in dose-related manner on both seventh day and fourteenth day compared with control group and diabetic group. Aloe vera at doses of 100 and 300 mg/kg daily for 4 days effectively decreased hydrocortisone's antiwound healing properties (Davis et al., 1994). It was found that Aloe vera stimulated fibroblasts to increase in number in a dose-response fashion. These findings would tend to indicate strength of wounds by producing collagen of the fibroblasts (Danof, 1987; Heggers et al., 1993; Winter et al., 1981). The ethanol precipitate which had mannose as major carbohydrate (Gowda, 1979) decreased the wound diameter by an average of 47.1% reduction (Davis, 1991). The growth factors including **giberellin, auxins** and **mannose-6-phosphate**, which were identified in Aloe vera, were effective in promoting wound healing (Davis et al., 1994; Morgan et al., 1987).

In a study of twenty-seven patients with partial thickness burn wound, they were treated with Aloe vera gel compared with vaseline gauze. It revealed the Aloe vera gel treated lesion healed faster than the vaseline gause

area. The average time of healing in the Aloe vera gel was 11.89 days and 18.19 days for the vaseline gauze treated wound. In histologic study, it showed early epithelialization in the treated Aloe vera gel area. Only some minor adverse effects, such as discomfort and pain were encountered in the 27 cases.

Somboonwong et al.(2000) demonstrated the microcirculatory and wound healing effects of Aloe vera on induced second degree burn wounds in rats. On seventh day, the vasodilation and increased postcapillary venular permeability as encountered in the untreated burn were found to be reduced significantly in both the NSS- and Aloe vera- treated groups, but to a greater extent in the latter. Leukocyte adhesion was not different among the untreated, NSS- and Aloe vera- treated groups. On fourteenth day, vasoconstriction occurred after the wound had been left untreated. Only in the Aloe vera- treated groups was arteriolar diameter increased up to normal condition and postcapillary venular permeability was not different from the sham control. The amount of leukocyte adhesion was also less observed compared to the untreated and NSS- treated group. Besides, the healing area of the Aloe vera-treated wound was better than that of the untreated and NSS-treated groups during seventh and fourteenth days after burn.

4. **Immunomodulation**

Acelylated mannans from aloe injected subcutaneously onto myelo suppressed mice stimulated and increased in white blood cell counts, splenic cellularity, and absolute numbers of neutrophils, lymphocytes and monocytes (Egger et al.; 1996, Davis et al., 1987; Davis et al., 1989). Aloe extracts reduced the production of interleukin-10 following exposure to ultraviolet radiation, reducing the suppression of delayed type hypersensitivity (Byeon et

al., 1998; Chong et al., 1997; Strickland et al., 1994). Aloe enhanced the antiinflammatory activity of hydrocortisone while blocking its wounding inhibition when applied topically to mice (Davis 1991,1994). Aloe extracts had antiinflammatory effects equivalent to hydrocortisone in the mouse ear model; although hydrocortisone administration was associated with a decrease in thymus weight, the aloe extracts had no such effect (Hutter et al., 1996). In rat paw models, fresh aloe gel showed significant antiflammatory activity and increased wound strength (Udupa et al., 1994; Chuhan et al., 1998). Rat with adjuvant-induced arthritis exhibited fewer symtoms when treated with a topical preparation containing aloe (Davis, 1985). Aloe extracts also blocked mast cell inflammatory response to antigen-antibody complexes (Ro et al., 1998, Yamomoto et al., 1993). In a case series of 14 HIV-1+ patients who were prescribed 800 mg/day of acemannan, there was a significant increase in the number of circulating monocyte and macrophages which mirrored clinical improvements. In a pilot study in HIV-infected persons acemannan increased the number of white blood cells and improved symptoms (MacDaniel et al., 1990). Aloe extracts also increased phagocytosis in asthmatic adults (Shida 1985).

OBJECTIVE OF THIS STUDY

The aim of this study are:

1. To study the effects of Aloe vera on wound healing area.

2. To study the effects of Aloe vera on changes of microcirculation as follows:
   - 2.1 tissue perfusion.
   - 2.2 arteriolar diameter.
   - 2.3 leukocyte adhesion.

3. To study the effects of Aloe vera on serum TNF-α and IL-6 levels.