ผลของสารสกัดดายขัด (*MALVASTRUM COROMADELIANUM* LINN.) ต่อการสมานแผลไหม้ระดับที่สองในหนูขาวที่เป็นและไม่เป็นเบาหวาน

นางสาวคันธรส สุขกุล

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EFFECTS OF *MALVASTRUM COROMADELIANUM* LINN. EXTRACT ON SECOND DEGREE BURN WOUND HEALING IN DIABETIC AND NON-DIABETIC RATS

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การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษา ผลของสารสกัดดายขัดด้วยน้ำต่อการสมานแผลไหม้ระดับที่สองในหนู Wistar เพศผู้ น้ำหนักระหว่าง 250-300 กรัม ทั้งที่ได้รับและไม่ได้รับการฉีด streptozotocin ในขนาด 60 มิลลิกรัมต่อกิโลกรัม เข้าทางหลอดเลือดดำที่หางเพื่อเหนี่ยวนำให้เป็นเบาหวาน แผลไหม้ถูกทำให้เกิดขึ้นโดยการใช้ แผ่นร้อน อุณหภูมิ 90 องศาเซลเซียส นาบที่หลังหนู เป็นเวลา 10 วินาที ให้สารทดสอบโดยการทาบริเวณแผลวันละหนึ่งครั้ง และประเมินผลโดยการสังเกตลักษณะภายนอกของแผล คำนวณระดับการหายของแผล ศึกษาการไหลเวียนเลือดที่ผิวหนังโดยใช้ Laser Doppler Flowmeter วัดระดับการเกิดลิปิดเพอร์ออกซิเดชันและศึกษาการเปลี่ยนแปลงทางจุลพยาธิวิทยา ในวันที่ 3, 7 และ 14 หลังการทำให้เกิดแผลไหม้

ผลการทดลองพบว่า สารสกัดดายขัดมีฤทธิ์ช่วยเร่งกระบวนการสมานแผลทั้งในหนูกลุ่มที่เป็นและไม่เป็นเบาหวาน โดยพบว่า ใน 3, 7 และ 14 วัน หลังเกิดแผลไหม้ ขนาดแผลในหนูทั้งสองกลุ่มที่ได้รับสารสกัดดายขัดจะมีขนาดลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูชนิดเดียวกันที่แผลไม่ได้รับการรักษา หรือกลุ่มที่ทาแผลด้วยน้ำเกลือ นอกจากนี้พบว่า สารสกัดดายขัด มีฤทธิ์เพิ่มการไหลเวียนของเลือดที่ผิวหนัง โดยพบว่า หนูกลุ่มเบาหวานที่ได้รับสารสกัดดายขัด มีค่าเฉลี่ยของการไหลเวียนของเลือดที่ผิวหนังเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติตลอดการทดลอง ในขณะที่จะพบการตอบสนองดังกล่าวในหนูที่ไม่เป็นเบาหวานในวันที่ 3 และ 7 เท่านั้น จากการสังเกตลักษณะของแผลและการเปลี่ยนแปลงทางจุลพยาธิวิทยา พบว่า สารสกัดดายขัดจะทำให้แผลในหนูกลุ่มที่ไม่เป็นเบาหวานปิดสนิทในวันที่14 ในขณะที่แผลในหนูกลุ่มเบาหวานจะยังปิดไม่สนิทแต่จะพบว่าเริ่มมีการปิดของผิวหนังชั้นหนังกำพร้าแล้ว ระดับเม็ดเลือดขาวชนิดนิวโทรฟิลบริเวณแผลจะลดลงในกลุ่มที่ได้รับสารสกัดดายขัดทั้งในหนูที่เป็นและไม่เป็นเบาหวาน นอกจากนี้ ยังพบว่าสารสกัดดายขัดมีฤทธิ์ลดการเกิดลิปิดเพอร์ออกซิเดชันซึ่งจะเห็นได้อย่างชัดเจนในวันที่ 3 และ 7 ในหนูกลุ่มที่เป็นเบาหวาน และในวันที่ 14 ในหนูกลุ่มเบาหวาน

การศึกษานี้แสดงให้เห็นว่าสารสกัดดายขัดมีฤทธิ์เร่งการสมานแผลไหม้ระดับที่สองในหนูขาวทั้งในกลุ่มที่เป็นและไม่เป็นเบาหวาน ถึงแม้ว่าการสมานแผลในหนูกลุ่มที่เป็นเบาหวานจะเกิดขึ้นได้ช้ากว่า ส่วนหนึ่งของฤทธิ์เร่งการสมานแผลดังกล่าวน่าจะเป็นผลจากการที่สารสกัดดายขัดสามารถเพิ่มปริมาณการไหลเวียนของเลือดบริเวณผิวหนังที่เกิดแผล ยับยั้งกระบวนการอักเสบ และมีฤทธิ์ต้านอนุมูลอิสระซึ่งเกิดขึ้นในแผลไหม้

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KANTAROTE SOOKKUL: EFFECTS OF *MALVASTRUM COROMANDELIANUM* LINN. EXTRACT ON SECOND DEGREE BURN WOUND HEALING IN DIABETIC AND NON-DIABETIC RATS. THESIS ADVISOR: ASSOC. PROF. BOONYONG TANTISIRA, Ph.D. THESIS CO-ADVISOR: ASSOC. PROF. MAYUREE TANTISIRA, Ph.D., ASSOC. PROF. JURAIPORN SOMBOONWONG, M.D., M.Sc., pp 125)

The present study aimed to investigate the healing effect of aqueous extract of *Malvastrum coromandelianum* (MC) Linn. extract on second degree burn wound, generated by 10 second-application of 90°C hot plate on the selected area of the back of non-diabetic and diabetic rats. Male Wistar rats weighting 250-300 g were used and diabetes was induced by a single injection of streptozotocin in the dose of 60 mg/kg B.W. intravenously into tail vein. Evaluation of wound healing by an estimation of degree of wound healing, measurement of cutaneous blood flow by Laser Doppler Flowmeter, analysis of malondialdehyde (MDA) as indicator of oxidative stress and histological observation were made at day 3, 7 and 14 posts burning.

The results revealed that degree of wound contraction in both diabetic and non-diabetic rats treated with MC extract was significantly different from those observed in their respective untreated and NSS-treated groups at day 3, 7 and14 posts burning. In comparison to their respective control groups, tissue blood flow was significantly increased in MC extract-treated group at all three time points in diabetic rats whereas it was observed only at day 3 and 7 in non-diabetic group. On day 14, histological observation demonstrated a complete re-epithelialization resulting in complete closure of the wound in non-diabetic rats receiving MC extract while epithelialization in MC extract-treated diabetes rats, though better than its control groups, still incomplete. Application of MC extract seemed to reduce the migration of neutrophils in diabetic and non-diabetic rats though with a lesser extent in the former group. In non-diabetic, burn injury significantly increased oxidative stress which was counteracted by MC extract day 3 and 7. However, in diabetic rats antioxidant property of MC was evident only at day 14.

In conclusion, the present studies have demonstrated the wound healing effect of the aqueous extract of *Malvastrum coromandelianum* Linn. on burn wound in diabetic as well as non-diabetic rats. Slower rate of healing was noticed in diabetic rats. An increase of cutaneous blood flow, anti-oxidation property as well as inhibition of the inflammation process is likely to explain, at least, in part, the beneficial effect observed.

Field of study physiology

Academic year 2006

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LIST OF ABBREVIATION

ARDS	Adult respiratory distress syndrome
AVA	Arteriovenous anatomoses
BG	Blood glucose
bFGF	Basic fibroblast growth factor
B.W.	Body weight
°C	Degree Celsius
cm	Centimeter
cm^2	Square centimeter
DM	Diabetic mellitus
e.g.	Exempli gratia (for example)
EGF	Epidermal growth factor
et al.	et alii (and orther)
FFA	Free fatty acids
GDM	Gestational diabetes mellitus
g/kg	Gram per kilogram
g/L	Gram per liter
gm	Gram
µg	Microgram
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glucose
IGI	Impaired glucose tolerance
IL-1	Interleukin-1
i.p.	Intraperitoneal
i.v.	Intravenous
kg	Kilogram
L	Liter
LDF	Laser Doppler Flowmeter

LSD	Least significant different test
MC	*Malvastrum coromadelianum* (LINN.) Garcke
MDA	Malondialdehyde
mg/kg	Milligram per kilogram
ml	Milliliter
mm	Millimeter
MODS	Multiple organ dysfunction sysndrome
NIDDM	Non insulin dependent diabetic mellitus
nmol	Nanomolar
NSS	Normal Saline Solution
NUTR	Nutritve
PDGF	Platelet-derived growth factor
per se	Per second
ROS	Reactive oxygen species
rpm	Round per minutes
TGF-alpha	Transforming growth factor alpha
TGF-ß	Transforming growth factor beta
TIMP	Tissue inhibitor of metallo-protease
TNF-*a*	Tumor necrosis alpha
TGF-*a*	Transforming growth factor alpha
SC	Subcutaneous
S.E.M.	Standard error of the mean
SIRS	The systemic inflammatory response syndrome
sq m	Square meter
sq inches	Square inches
STZ	Streptozotocin
UV	Ultraviolet
VEGF	Vascular endothelial cell growth factor
v/v	Volume by volume
w/v	Wight by volume

CHAPTER I

INTRODUCTION

Thermal injury is more commonly induced in tissue by sudden application of excessive thermal energy. A thermal insult to the skin is followed by a dynamic response in microcirculation, inducing a pronounced release of numerous noxious vasoactive agents (Korthuis, Anderson and Granger, 1994; Mayers and Johnson, 1998). Vasodilatation and increased microvascular permeability cause rapid formation of local edema, followed by reduction in perfusion, impaired circulation, leading to local tissue ischemia (Kloppenberg, Beerthuizn and Ten Duis, 2000), which ultimately leads to several degrees of cellular dysfunction, local progressive skin necrosis and distant organ injury in burns (Till *et al*., 1989; Demling and LaLonde, 1990; Thompson *et al*., 1990).

Free radicals and their scavenging system are also known to play a very important role in healing of normal and delayed healing type of wound (Shula, Rasik and Patnaik., 1997; McDaniel *et al*., 1998). Therefore, it is possible that some kind of correlation exists between altered free radical cascades and delayed wound healing. Thus, any factors that could reduce lipid peroxidation (and consequently the malondialdehyde (MDA) formation, a stable end product of lipid peroxidation) might help promote the healing of burn injury (Mallikarjuna *et al*., 2002).

Optimum treatment of the wound reduces morbidity and mortality. It also shortens the time for healing and return of normal function and reduces the need for secondary reconstruction (Papini, 2004). Factors affecting wound healing include wound care, good nutrition, improvement of the blood supply of partial-thickness burns, prevention of burn wound infection, reduction of wound edema formation (Cioffi, 2001; Sim, 2002), associated illness such as diabetes mellitus (DM) (Napoli *et al*., 1999; Blakytny and Jude, 2006).

Diabetes mellitus is a chronic metabolic disorder that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro- and microvascular dysfunctions (Duckworth, 2001). Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. It is accepted that oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential (Abdollahi *et al*., 2004). It is evident that hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in variety of tissues (Evans *et al*., 2002). In both insulin dependent (type1) and non-insulin dependent diabetes (type2) there is increased oxidative stress (Nazirogilu and Butterworth, 2005).

Abnormal cellular functioned, particularly of fibroblast and neutrophil, absence of cellular growth and migration of epidermis over the wound, together with narrowing or occlusion of the blood vessels within the edge of the wound (Ferguson *et al*., 1996). Delayed wound healing and ulcer formation in diabetes us associated with poor local blood supply, infection, callus formation (Ehrlichman *et al*., 1991; Jeffcoate and Harding, 2003) and the effect of hyperglycemia (insulin insufficiency or resistance) (Napoli *et al*., 1999).

Cutaneous thermal wound management is still a great problem. Burned wound care is needed according to the severity of burn. The concepts of optimum minor burn wound management remained focused on avoiding wound infection, generally treated with topical antibiotics agent or ointment, occlusive dressing (closed method) and wet dressing (generally treated with normal saline solution) since epithelialization progresses fasten in a moist environment. While severe burns are considered an emergency, requiring hospitalization. These injuries are most difficult to assess and treat. (Heimbach, Mann and Engrav, 1996; Papini, 2004)

Even in developed countries, more than 2 million individuals annually are burned seriously and required medical treatment (Levy and Moskowitz, 1982). Topical antibiotics are used routinely in the forms of antimicrobial creams e.g. silver sulphadiazine (Flamazine) applied locally to injury skin after cleaning.

Silver sulphadiazine (Fox et al., 1968), is thought to act via inhibition of DNA replication and modifications of the cell membrane and cell wall. It is used for prevention and treatment of infection in second-and third-degree burns. However, treatment falls with continued use in large burns (> 50 % TBSA). Though it is a broad spectrum antimicrobial, it has the disadvantage of not being absorbed through eschar. Thus, it is not effective in the wound that has eschar or infection. The side effects of this agent are rash and transient leukopenia. Due to bone marrow suppression, the use of silver sulphadiazine in G6PD patient may cause hemolytic anemia (Lodah, Samplh and Fox 1988; Noronha and Almeida, 2000; Chaiyaphruk, 2003).

Despite many advanced medical treatments, we still have to encounter the side effects and high expense. Thailand has many kinds of herbal products which have long been used in traditional medicine; the government has tried to build up self-reliance on drug supply by promoting the uses of traditional medicine. Medicinal plants are recommended to be grown and used in the households and community hospitals. *Malvastrum coromadelianum (L.)* Garcke (MC) or “Dai-Kat” or “Ya-Tevada” in Thai is one of medicinal plants scientifically investigated by the Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Health of Thailand. Common name is Prickly Malvastrum. MC, belonging to Malvastrum family, It is perennial or annual (sometimes), broad-leaved, erect herbs or shrubs (sub shrub), up to 0.5 (-1.5) m high. It is Thai medicine plant that has been claimed to treat diabetes mellitus.

Rattanajarasroj *et al*., (2004) reported that MC-1 extract (a portion of water extract MC showed a significant hypoglycemic activity in alloxan-induced diabetic rats. It significantly reduced blood sugar levels lower than those of the control, after an oral

administration, respectively. Jesadanont et al., (2005) investigated that water extract from whole plant of MC extract showed strong hypoglycemic activity when administered orally. Pongpech *et al*., (2005) showed that water extract from whole plant of MC extract had an inhibitory effect against *Staphylococcus aurous* when tested in vitro. MC was investigated by Moulun *et al*, (1999) for its effects on fever caused by typhoid-parathyroid vaccine in rabbits, hot-plate, writhing, ear edema, and abdominal capillary permeability of mice. Result of the study showed that both aqueous extract of whole plant or root of MC extract could lower rabbit fever, decrease mice writhing, inhibit ear edema and decrease the permeability of blood capillary in abdominal cavity of mice. These results have suggested that MC has antipyretic, analgesic and anti-inflammatory effects.

Attawish *et al*., (1998) studied that chronic toxicity study of water extract of MC given orally to the animals for six-month period. Histopathological study of internal organ did not reveal any abnormalities that could be attributed to the toxicity of extract.

As mention above, some studies have demonstrated that MC extract has hypoglycemia, antibacterial, antipyretic, analgesic and anti-inflammatory effects. In previous studies MC extract was given by oral administration. In topical administration isn't report. Furthermore, there are no studies that have been carried out on burn wound model especially in diabetes. Thus, the purpose of the present study was to investigate the effects of the extract from MC on wound healing in burn wound model and burn wound in diabetes and non-diabetic.

Objective

1. To study the effects of MC extract on second degree burn wound healing in diabetic and non diabetic rats.

CHAPTER II

LITERATURE REVIEWS

2.1 The Skin

The skin is one of the largest organs of the body in terms of surface area. For the average adult, the skin occupies a surface area of approximately 2 sq m (3,000 sq inches), weights approximately 3.2 kg and receives about one-third of all blood circulating through the body. The skin is quite complex in structure and performs several functions essential for survival.

2.1.1 Structure of the skin

Structurally, the skin consists of three principal parts. The outer, which is composed of epithelium, is called the epidermis, connective tissue part called the dermis. Beneath the dermis is subcutaneous (SC) layer. This layer, also called the superficial fascia or hypodermis, consists of areolar and adipose tissue as show in Figure2.1.

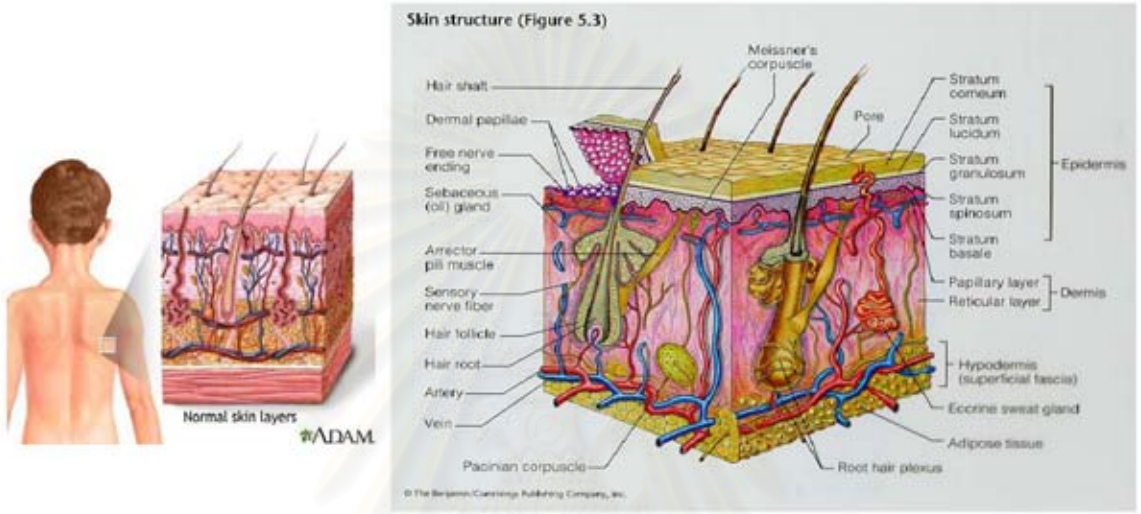


Figure 2.1 The skin (Fuchs and Byrne, 1994)

2.1.1.1 Epidermis

The outer, or epidermis layer of the skin composed of stratified squamous epithelial cells. The most numerous one is known as a *keratinocyte*, a cell that undergoes keratinization. The functions of these cells are to produce the protein keratin. A second type of cell in the epidermis is called a *melanocyte*. It is located at the base of the epidermis, and its role is to produce melanin, one of the pigments responsible for skin color and the absorption of ultraviolet (UV) light

The epidermis has five layers, from superficial to deep, stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum germinativum. This layer consists of 25 to 30 rows of flat, dead cell completely filled with keratin. These cells are continuously shed and replaced. The stratum corneum serves as an effective barrier against light and waves, bacteria, and many chemicals. The stratum lucidum (clear

layer) is normally found only in the thick skin of the palms and sole and is absent in thin skin. It consists of three to five rows of clear, flat, dead cells that contain droplets of a substance called eleidin. The stratum granulosum (granular layer) is the third layer of epidermis consists of three to five rows of flattened cells that contain darkly staining granules of substance called keratohyalin. This compound is involved in the first step of keratin formation. The stratum spinosum (prickly layer) contains 8 to 10 rows of polyhedral cells. The surfaces of these cells contain spinelike projections that join the cells together. The last layer is stratrum basal or stratum germinativum (regenerative layer) consists of cuboidal to columnar cells with the capability of continuing cell division. As these cells multiply, they push up toward the surface and become part of the other layers. Other cells in the stratum basal migrate into the dermis and give rise to sweat and oil glands and hair follicles. The stratum basal of hairless skin contains nerve endings sensitive to touch called tactile (Markel's) discs (Stanley, 1982; Tortora and Anagnostakos, 1990; Falkel, 1994).

2.1.1.2 Dermis

The second principal part of the skin, the dermis, composed of connective tissue containing collagenous and elastic fiber. Numerous blood vessels, nerves, glands, and hair follicles are embedded in the dermis. The upper region of dermis is named the papillary region or layer. It consists of loose connective tissue containing fine elastic fibers. The reticular layer is made up of dense connective tissue with coarse collagenous fiber and fiber bundles that crisscross to from strong and elastic network. The cells of the dermis are mostly fibroblasts, fat cells and macrophages. Blood vessels, lymphatic vessels, nerve endings, hair follicles and glands are also present (Mast, 1992).

2.1.1.3 Hypodermis

The hypodermis (subcutaneous) is beneath the dermis and composed of loose, fibrous, connective tissue. The hypodermis is generally much thicker than the dermis and is richly supplied with lymphatic, blood vessels and nerves. Also within the hypodermis are the coiled ducts or sudoriferous (sweat) gland and the base of hair follicles. The boundary between the epidermis and dermis is distinct but that between the dermis and the hypodermis is not.

2.1.2 Function of the skin

Function of the skin are to protect us from heat, light, injury, infection and regulate body temperature; store water, vitamin D and fat; help sense pain and other stimuli and prevent the entry of bacteria (University of Virginia Health System, 2006; University of Maryland Medical Center; Mcnees, 2006)

2.2 The wound and wound healing

2.2.1 Definition of wound

A wound is defined as the loss of continuity of epithelium, with or without loss of underlying connective tissue (including muscle, bone and nerves for example), following injury. The injury may follow direct violence or be inflicted by non-mechanical injury which may also be responsible for delay in healing. Extensive tissue damage e.g., contusion or hematoma could occur with minimal tissue loss (Leaper and Gottrup, 1999).

2.2.2 Wound healing

2.2.2.1 Process of wound healing (Figure 2.2)

Wound healing is a physiologic process involving a series of sequential yet overlapping stages. There are anywhere from 3 to 5 stages of wound healing. The first stage, hemostasis, occurs immediately at the time of injury and is usually completed within hours. The second stage, inflammation, begins shortly after hemostasis and is usually completed within the first 24 to 72 hours after injury (Hass, 1995). However, it may last as long as 5 to 7 days after injury. The third stage of proliferation and repair typically occurs 1 to 3 weeks after injury. The fourth and final stage, remodeling, begins approximately 3 weeks after injury and may take anywhere from months to several years to achieve physiologic completion. (Waldrop and Doughty, 1991; Cooper, 1999; Mast, 1999; Ressell, 1999)

2.2.2.1.1 Stage 1: Hemostasis

Tissue injury causes the disruption of blood vessels and extravasation of blood constituents. The first step in wound healing is thus hemostasis (Kirsner and Eaglstein, 1993), which is initiated by variety of factor. As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing (Clark, 2001). Platelets not only release the clotting factors but they also provide a cascade of chemical signals, known as cytokines or growth factors, that initiate the healing response. The one most important signal is platelet-derived growth factor (PDGF) and initiates the chemotaxis of neutrophils, macrophages, smooth muscle cells and fibroblasts. In addition, it also stimulates the mitogenesis of the fibroblasts and smooth muscle cells. The cellular response of the inflammatory phase is characterized by the influx of leucocytes into the area of injury.

2.2.2.1.2 Stage 2: Inflammation

The second stage of wound healing is inflammation. Neutrophils are the next predominant cell marker in the wound within 24 hours after injury. The major function of the neutrophil is to remove foreign material, bacteria and non-functional host cells and damaged matrix components that may be present in the wound site (Hart, 2002; Sylvia, 2003). Bacteria give off chemical signals, attracting neutrophils, which ingest them by the process of phagocytosis.

The mast cell is another marker in wound healing. Mast cells release granules filled with enzymes histamine and other active amines. These mediators are responsible for the characteristic signs of inflammation around the wound site (Artuc *et al*, 1999). The active amines released from the mast cell, cause surrounding vessels to become leaky and thus allow the speedy passage of the mononuclear cells into the injury area. In addition, fluid accumulates at the wound site and the characteristic signs of inflammation begin. The signs of inflammation have been well recognized since ancient times: *rubor* (redness), *calor* (heat), *tumor* (swelling) and *dolor* (pain).

By 48 hours after injury, fixed tissue monocytes become activated to become wound macrophages. These specialized wound macrophages are perhaps the most essential inflammatory cells involved in the normal healing response (Diegelmann, Cohen and Kaplan 1981). Inhibition of macrophage function will delay the healing response (Leibovich and Ross, 1975). Once activated these wound macrophages also release PDGF (Platelet-Derived Growth Factor) and TGF-ß (Transforming Growth Factor-ß) that further attracts fibroblasts and smooth muscle cells to the wound site. These highly phagocytic macrophages are also responsible for removing nonfunctional host cells, bacteria-filled neutrophils, damaged matrix, foreign debris and any remaining bacteria from the wound site. Neutrophils phagocytes debris and bacteria and also kill bacteria (Greenhalgh, 1998; Muler, *et al*., 2003). The presence of wound macrophages is a marker that the inflammatory phase is nearing an end and that the proliferative phase is beginning.

2.2.2.1.3 Stage 3: Proliferation and Repair

The proliferative phase is characterized by angiogenesis; collagen deposition, granulation tissue formation, epithelialization, and wound contraction (Midwood et al, 2004)

Neovascularization

Local factors in the wound microenvironment such as low pH, reduced oxygen tension and increased lactate actually initiate the release of factors needed to bring in a new blood supply (Lavan and Hunt, 1990). This process is called angiogenesis or neovascularization and hypoxia induces macrophages into secreting angiogenic growth factor (vascular endothelial cell growth factor, VEGF, basic fibroblast growth factor (bFGF) and TGF-ß) (Battegay, 1995; Tonnesen, Feng and Clark, 2000).

Re-epithelialization

As the proliferative phase progresses, the process of epithelialization is stimulated by the presence of EGF (epidermal growth factor) and TGF-*a* (transforming growth factor *a*) that are produced by activated wound macrophages, platelets and keratinocytes (Yates, 1991; Schltz et al, 1991; Hunt et al, 1984). Keratinocytes migrate cover the surfaces of the skin defect. The keratinocytes proliferate and migrate across the wound. Once migration is complete, the keratinocytes stabilize themselves by forming firm attachments to each other and the new basement membrane (Clark, 1995; Garrett, 1997). The skin surface is completely covered with new epidermal cells, the wound is closed.

Granulation

Final mechanism of proliferative phase is progresses of granulation tissue; the predominant cell in the wound site is the fibroblast. Fibroblasts attach to the cables of the fibrin matrix and begin to produce collagen (Clark, 2001). At least 23 individual

types of collagen have been identified to date but type I is predominant in the scar tissue of skin (Prockop and Kivirikko, 1995). The collagen molecule begins to form its characteristic triple helical structure and the nascent chains undergo further modification by the process of glycosylation (Blumenkrantz *et al*, 1984). The procollagen molecule is then secreted into the extracellular spaces where it is further processed (Prockop *et al*, 1998). Hydroxyproline in collagen is important because it gives the molecule its stable helical conformation (Zanaboni *et al*, 2000). Finally, the extra-cellular spaces matrix, acts on the collagen to form stable cross-links. At 3 weeks after injury, the healing wound has approximately 20% of its final strength (Waldrop and Doughty, 1991; Iocono *et al*, 1998).

2.2.2.1.4 Stage 4: Remodeling

Finally, in the process of collagen remodeling or maturation of the granulation tissue, collagen degradation also occurs (Pilcher *et al*, 1999; Parks, 1999). Remodeling consists of deposition of the matrix and its subsequent change over time. In contraction, the wound is made smaller by action of myofibroblast and the wound begins to contract and the collagen matures. This important cross-linking step gives collagen its strength and stability over time (Hornstra *et al*, 2003). Dermal collagen on a per weight basis approaches the tensile strength of steel; in normal tissue it is a strong and highly organized molecule. In contrast, collagen fibers formed in scar tissue are much smaller and have a random appearance; scar tissue is always weaker and will break apart before the surrounding normal tissue. The regained tensile strength in a wound will never approach normal. In fact the maximum tensile strength that a wound can ever achieve is approximately 80% of normal skin (Diegelman and Evans, 2004; Jei Li, Che and Kirsner, 2007)

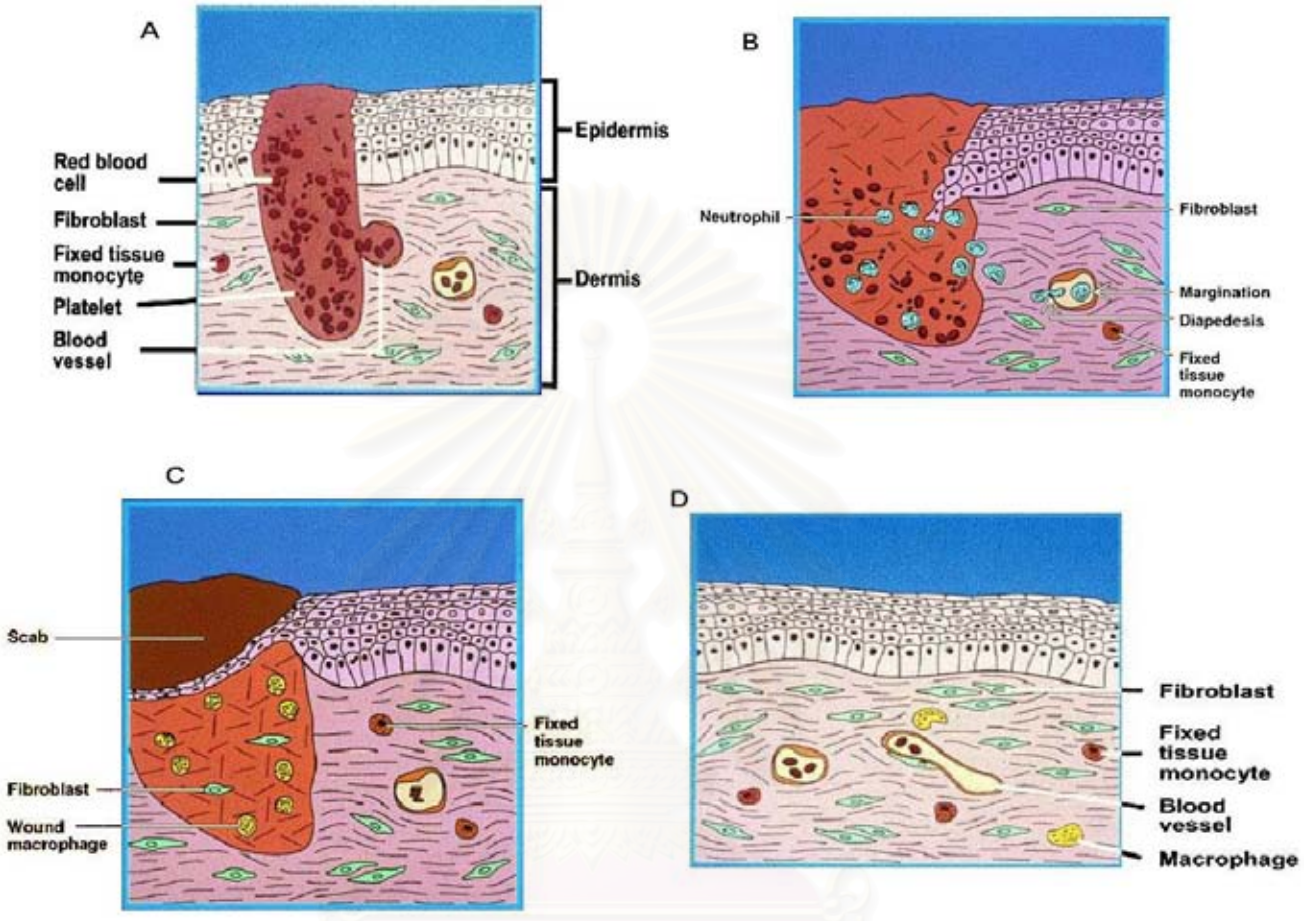


Figure 2.2 The wound healing, A: At the time of injury, the tissue is disrupted and the platelets adhere and release clotting factors, PDGF and TGF- ß to initiate the repair process. B: By the first day following injury, neutrophils attach to endothelium cells in the vessel walls surrounding the wound (migration), then change shape to move through the cell junction (diapedesis) and migrate to the wound site (chemotaxis). This is the beginning of the inflammatory phase. C: The inflammatory phase continues as fix tissue macrophages become activate and move into the site of injury and transform into very active wound macrophages. These highly phagocytic cell also relase PDGF and TGF-ß to recruit fibroblasts to the site and begin the proliferative phase. D: The remodeling phase is characterized by continued synthesis and degradation of the extracellular matrix component trying to establish a new equilibrium (Jei Li, Che and Kirsner, 2007)

2.2.3 Type of wound healing

2.2.3.1 Primary healing (healing by first intention, Figure 2.3)

Occur when a wound is closed within 12-24 hours of its creation (e.g. clean surgical incision, clean laceration). The wound edges are approximated closed directly by sutures, tissue glue, tapes or a mechanical device.

The incision causes only focal disruption of the continuity of the epithelial basement membrane (BM), and the death of relatively few epithelial and underlying connective tissue cells. As a result, epithelial regeneration predominates over fibrosis. Also, because there is appropriate balance between all phases of the healing process (including cellular proliferation, collagen metabolism, activity of matrix metalloproteinase, degradation of the extracellular matrix), wounds heal well and proceed rapidly towards complete closure.

2.2.3.2 Secondary healing (healing by second intension, Figure 2.3)

Occur in a wound with extensive loss of soft tissue, as seen in major trauma, severe burns and after some surgical procedures (e.g. laparostomy). Regeneration of epithelial cells alone cannot restore the original architecture, so there is ingrowths of granulation tissue from the wound margin, followed by accumulation of extracellular matrix with the laying down of collagen. These open, full-thickness wounds thus close by subsequent wound contraction and epithelialization. Healing by secondary intention is slower, may lead to contractures (particularly over joints) and functional restriction (Cotran, Kumar and Robbins, 1994;; Kumar, Cotran and Robbins, 2003)

2.2.3.3 Healing of superficial (partial-thickness) wounds is seen in injuries such as superficial burns, split-thickness donor graft sites and abrasions where the injury involves the epithelium and superficial (papillary) part of the dermis. The basal layer of cell remains uninjured, and sebaceous glands replicate to cover the exposed dermis; the cells migrate towards each other from the basal layer to surround the wound.

Healing occurs purely by epithelialization, and anatomical and physiological restoration is virtually complete (Greenhalgh and Staley, 1994).

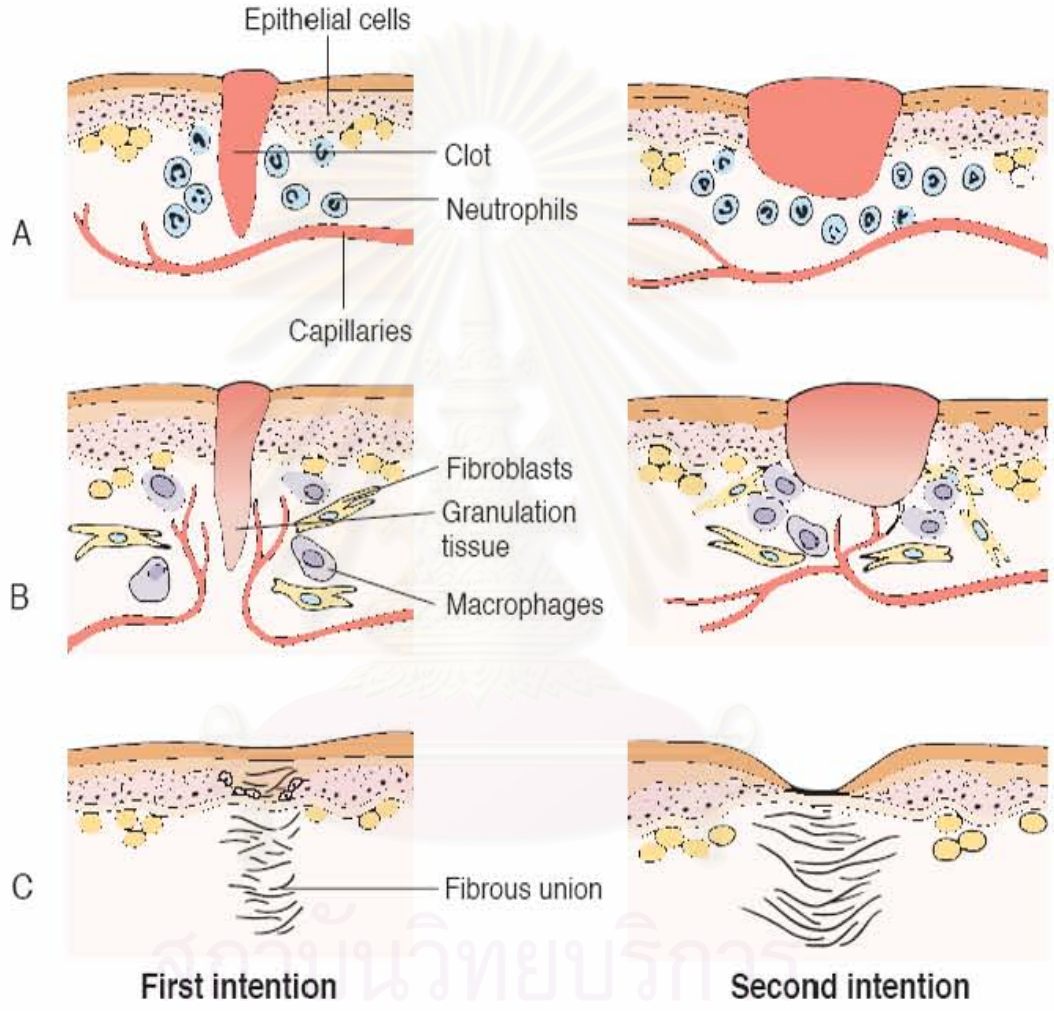


Figure 2.3 Steps in wound healing by first intension (*left*) and second intension (*right*). In the latter, the resultant scar is much smaller than the original wound, owing to wound contraction. (Greenhalgh and Staley, 1994))

2.3 The burn wound and wound healing

2.3.1 Burns

Tissues may be damaged by thermal (heat), electrical, radioactive, or chemical agents, all of which can destroy (denature) the proteins in the exposed cell and cause cell injury or death. Such damage is a burn. Burns disrupt homeostasis because they destroy the protection afforded by the skin, allowing microbial invasion and infection, loss of fluid, and loss of temperature control. The systemic effects of a burn may include a large loss of water, plasma and plasma protein, which causes shock, bacterial infection, reduced circulations of blood and decrease production of urine. However, the magnitude of involvement of these functions depends on the extension and depth of the burn (Lee and Astumian, 1996; Sheridan, 2003).

2.3.2 Mechanisms of burn

2.3.2.1 Thermal burns

The local thermal wound is the result of heat necrosis of cells. The content of cellular destruction depends upon several factors the intensity of heat tissue involved. The conductance of the tissue involved determines the rate of dissipation or absorption of heat and depends upon several factors. These include the peripheral circulation, water content of the tissue, thickness of the skin and its pigmentation of the presence or absence of external insulting substances such as hair and skin oil. Of these factors perhaps the most important in determining the degree of injury is the peripheral circulation (Artz and Yarbrough, 1970). The rate of blood flow through the heat exposed tissues can be altered rapidly. This mechanism is of major importance in determining the amount of cellular destruction associated with the transfer of heat to the tissue. Thermal burns may be further subdivided into flame burns, flash burns, scald burns and contact burns (Latha and Babu, 2001).

2.3.2.2 Electrical burns

Electrical injuries result from the heat produced by the flow of electrical current through the resistance of body tissues. Factors of primary importance in determining the effect of the passage of an electric current through the human body include the type of circuit, voltage, resistance of the tissues involved, the path of the current through the body and duration of contact with the current. The chief reason for considering these wounds as a category distinct from the more common thermal burn is the volume of tissue that is often involved in high voltage electrical injuries.

2.3.2.3 Chemical burns

A wide variety of agents may be responsible for chemical burns. The majority of chemical agents produce skin destruction through chemical reactions rather than hyperthermia injury (Jelenko, 1970 and Jelenko, 1974). Included among these reactions are coagulation of protein by reduction, corrosion, oxidation, formation of salts, poisoning of protoplasm and desiccation. Acids promote collagen denaturation and subsequent degradations. Heat production is often a by-product of the chemical reactions with tissues and may worsen the injury (Hettiaratch and Dziewulski, 2004).

2.3.3 Pathophysiology of thermal burn

2.3.3.1 Local and systemic response

The pathophysiological changes in the burn wound are characterized by effects caused by heat *per se* and superimposed on. These are a pronounced acute inflammatory process (Kramer, Lund and Herndon, 2002). A sudden increase in body surface temperature results in prompt local 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 reaction then take place. The inflammatory mediators which control blood supply and microvascular permeability in wound have been extensively studied and are largely understood. Prostaglandins, thromboxanes and leukotrienes are produced through the arachidonic cascade.

The inflammatory responses to injury, infection and antigen challenge with overproduction of chemical mediators, activation of leukocytes and endothelial cell and alteration in circulating cytokine may all contribute to system effects. Thus in patients with major burns these effects are: increased susceptibility to infection, the systemic inflammatory response syndrome (SIRS), adult respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), which may develop further into progressive organ failure and death. (Arturson, 1996; Somboonwong and Duansak; 2004; Burd and Chiu, 2005)

2.3.3.2 Postburn change

Usually the burn wound initially has different depths in different regions. There are three degrees of burns, the first in which only the epidermis is damaged, the second where some dermal changes also occur but where epithelial regeneration is possible and the third where both epidermis and dermis are irreversibly damaged (Bunyapraphatsara, 1996). Often the wound is characteristically made up of several zones of tissue damage due to different heat transfer (Jackson and Mac, 1983; Arturson, 1996). In the middle, usually the site of greatest heat transfer, irreversible skin death occurs, resulting in the zone of coagulation. The 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. (Arturson, 1996; Somboonwong and Duansak; 2004)

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 hr postburn due to vasodilation, increased extravascular osmotic activity** (Arturson and Mellander, 1964) **and increased microvascular permeability** (Arturson, 1961; Arturson, 1979, Nozaki *et al*., 1979). A rapid degradation of hyaluronate and collagen fiber 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 interstitum due to this so-called imbibitions pressure (Lund *et al*., 1989) is then further accentuated by fluid leakage due to increased microvascular permeability.

2. These changes are followed by heterogeneous reduction 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 favor adhesion on the free surfaces of endothelial cells, platelet and leucocytes** (Von Andrian *et al*., 1991). This leads to leucocyte margination followed by extravasation and their migration to the injury parenchymal cells and microorganisms. Platelets removed from the circulation contribute at different levels to haemostasis and local thrombosis.

4. **A later phase of wound repair** with high rates of wound perfusion to support wound metabolic requirements and maintain adequate defense against invasive burn wound infections (Jackson and Mac, 1983).

5. **Burn wound microbial colonization and infection**. Gram-positive bacteria in the depths of hair follicles and sweat glands may heavily colonized the wound within the first 48 hours postburn, especially if topical chemotherapy is not applied. The microorganisms present in the wounds of hospitalized patients change with time after injury. Usually gram-positive organisms (Staphylococcus aureus, Streptococcus pyogenes) during the first week postburn are superseded by gram-negative organisms (*Pseudomonas aeruginosa*, *Escherichia coli*) during the second week.

The first and most obvious difference between burn and disruptive or incisional wounds is in the effect of the injury upon blood vessels at the site of injury. Burn injury damages blood vessels both in the immediate area of the wound to cause restriction or cessation of blood flow at the site of injury and modification to blood flow in the surrounding area. The best description of this has been given by Zawacki (1974).

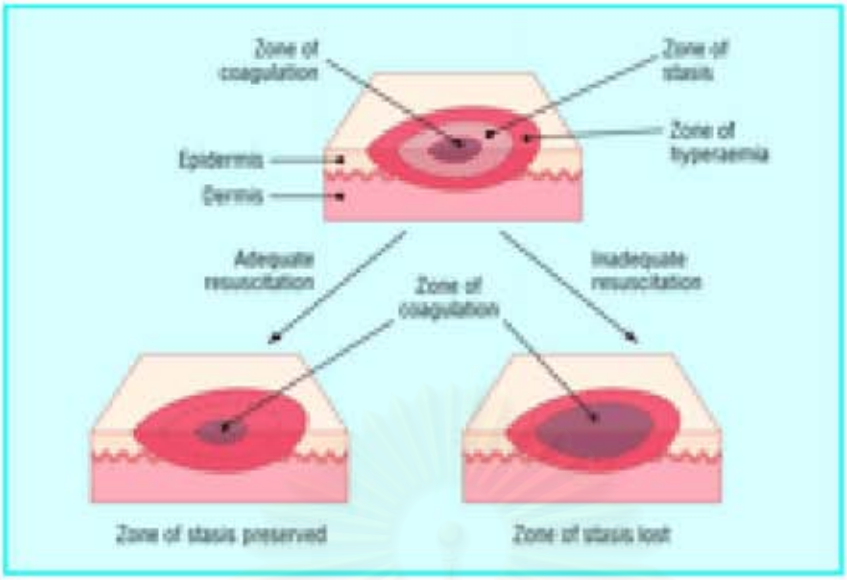


Figure 2.4 Jackson's burn zone (1994); **Zone of coagulation**-This occurs at the point of maximum damage. In this zone there is irreversible tissue loss due to coagulation of the constituent proteins. **Zone of stasis**-The surrounding zone of stasis is characterized by decreased tissue perfusion. The tissue in this zone is potentially salvageable. The main aim of burns resuscitation is to increase tissue perfusion here and prevent any damage becoming irreversible. Additional insults-such as prolonged hypotension, infection, or edema can convert this zone into an area of complete tissue loss. **Zone of hyperemia**-In this outermost zone tissue perfusion is increased. The tissue here will invariably recover unless there is severe sepsis or prolonged hypoperfusion (Hettiaratchy and Dziewulski, 2004).

2.3.4 Classification of burn depths (Bonaldi and Frank, 1987; Johnson, 1994)

An accurate determination of the depth of injury is an important consideration when formulating a management plan for a patient with burn (Sevitt, 1957; Foley, 1970). Burns are classified into two groups by the amount of skin loss. Partial thickness burns do not extend through all skin layers, whereas full thickness burns extend through all skin layers into the subcutaneous tissues. Partial thickness burns can be further divided into superficial, superficial dermal, and deep dermal (Hettiaratchy and Papini, 2004) (Figure 2.5).

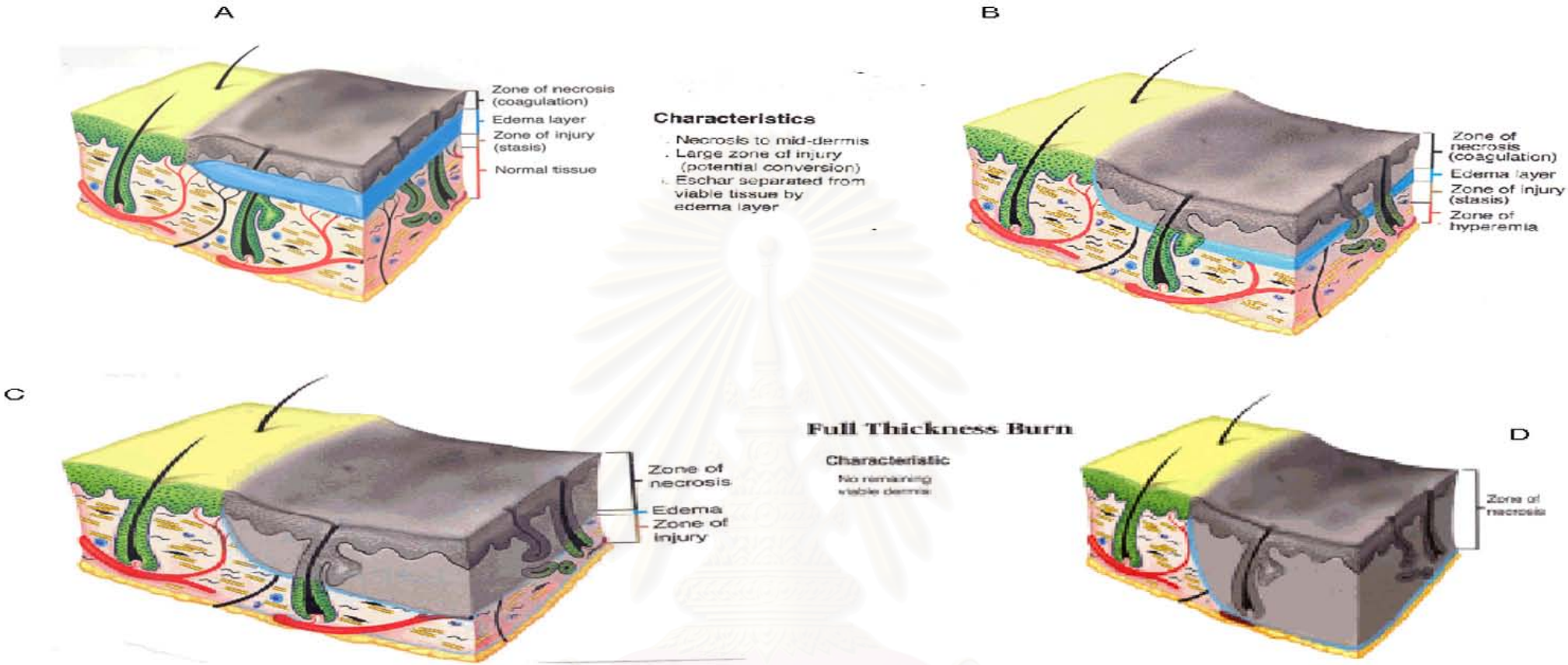


Figure 2.5 Classification of burn depths A: superficial or first-degree burn -The burn affects the epidermis but not the dermis (such as sunburn). It is often called an epidermal burn. B and C: partial-thickness or second-degree burns- The burn extends through the epidermis in to the upper layers of the dermis and is associated with blistering. D: full-thickness or third-degree burns- Involves destruction of epidermis, dermis, and the epidermal derivatives, and skin function loss.

2.3.4.1 Functional and descriptive classification

While the above traditional classification is still present, it is being rapidly abandoned for a function and descriptive classification that allows a more precise description of the burn wound. Burn wound are now classified into superficial, partial thickness, and full thickness wounds. The spectrum of both eventual outcome and treatment varies greatly from the superficial burns to those of full thickness. It is thus important to choose a grading system that will allow the cost complete understanding of the pathophysiological process taking place in the burn wound.

2.3.4.1.1 The superficial burn wound or First-degree burns

In the most superficial injuries, only the upper epidermis shows changes. These wounds are frequently the result of either prolonged exposure to low intensity heat, e.g., sunburn, or to a short duration flash exposure to a high intensity heat source. Erythema of the skin with edema confined to the basal layers is the result. Irritation of naked nerve ending occurs but swelling is not consistently present. In some cases, cell death at the level of the stratum granulosum does occur. This results in desquamation for two or three days following the burn and is recognized as the typical peeling following sunburn. There is no significant, early clinical consequence to burns at this level. The wounds heal rapidly without leaving a trace of scar. Late changes, such as an increased rate of neoplastic degeneration, are well recognized following solar and ionic radiation, but have not been documented for flash explosion burns.

2.3.4.1.2 The partial thickness burn wound or Second-degree burns

A superficial partial thickness burn is equivalent to the classic second degree blister burn. These injuries are the result of either increased exposure time or higher intensity flash exposure, and imply further cellular destruction than present in a simple superficial burn. The basal layer of the skin provides the line of demarcation between the deep and superficial partial thickness injury. In the superficial partial thickness (second degree) burn, the basal layer is not totally destroyed. As in the first degree

superficial partial thickness (second degree) burn, the basal layer is not totally destroyed. As in the first degree burn, erythema is a prominent feature, however, blistering is the hallmark of this level of burn. Cellular destruction of the stratum granulosum and stratum corneum occurs forming the covering of these blisters. The vascular response of the subpapillary plexus within the dermis results in edema formation at the dermal-epidermal junction. As fluid accumulates, the junction separates forming the blister. The epidermis itself may become swollen and edematous. Again, nerve endings are irritated and those can be extremely painful injuries.

The deep partial thickness burn consists of a wound with complete disruption of the epidermis and destruction of most of the basal layer. Sparing of dermal appendages such as hair follicles and sweat glands allows the wound to potentially regenerate and is thus partial thickness. The events that occur in the subpapillary plexus characterize the major histological changes in this injury. Edema fluid infiltrates the dermal-epidermal junction. Ischemic (coagulation) necrosis of the epidermis occurs, followed by an inflammatory cellular response incited at the basilar level resulting in further tissue destruction. Blistering may occur, however, this is not an essential component. The wound is more often characterized by eschar formation. Microhygrometer readings of deep partial thickness burn eschar demonstrate massive fluid loss. Of major clinical importance and a significant difference from the superficial burn is the loss of cellular barrier which protects against bacterial and wound sepsis.

2.3.4.1.3 The full thickness burn wound or Third-degree burns

In the full thickness injury the epidermis is destroyed along with dermal appendages and supporting structures. The wound is characterized by coagulation necrosis of cells and only at the edges of the wound do edema and cellular infiltrates occur.

2.3.5 Assessment of burn area (Hettiaratchy and Papini, 2004)

Assessment of burn area tends to be done badly, even by those who are expert at it. There are three commonly used methods of estimating burn area, and each has a role in different scenarios. When calculating burn area, erythema should not be included. This may take a few hours to fade, so some overestimation is inevitable if the burn is estimated acutely.

2.3.5.1 Palmar surface

The surface area of a patient's palm (including fingers) is roughly 0.8% of total body surface area. Palmar surface are can be used to estimate relatively small burns (< 15% of total surface area) or very large burns (> 85%, when unburn skin is counted). For medium sized burns, it is inaccurate.

2.3.5.2 Wallace rule of nines (Figure 2.6 A)

A body diagram and chart are used to estimate burn size. Commonly, the rule of 9s is used because it is easy to remember. This is a good, quick way of estimating medium to large burns in adults. The body is divided into areas of 9%, and the total burn area can be calculated. It is not accurate in children.

2.3.5.3 Lund and Browder chart (Figure 2.6 B)

This chart, if used correctly, is the most accurate method. It compensates for the variation in body shape with age and therefore can give an accurate assessment of burns area in children. It is important that all of the burn is exposed and assessed. During assessment, the environment should be kept warm, and small segments of skin exposed sequentially to reduce heat loss. Pigmented skin can be difficult to assess, and in such cases it may be necessary to remove all the loose epidermal layers to calculate burn size.

There are many factors involved in burns that must be observed in an assessment. Depth extension and burn localization, victim's age, existence of previous diseases, concomitance of aggravating considered when assessing burns. The assessment environment must be heated so as to minimize liquid loss in the skin through evaporation, as the skin has to be left uncovered and be examined in sections (Siviero, 2005).

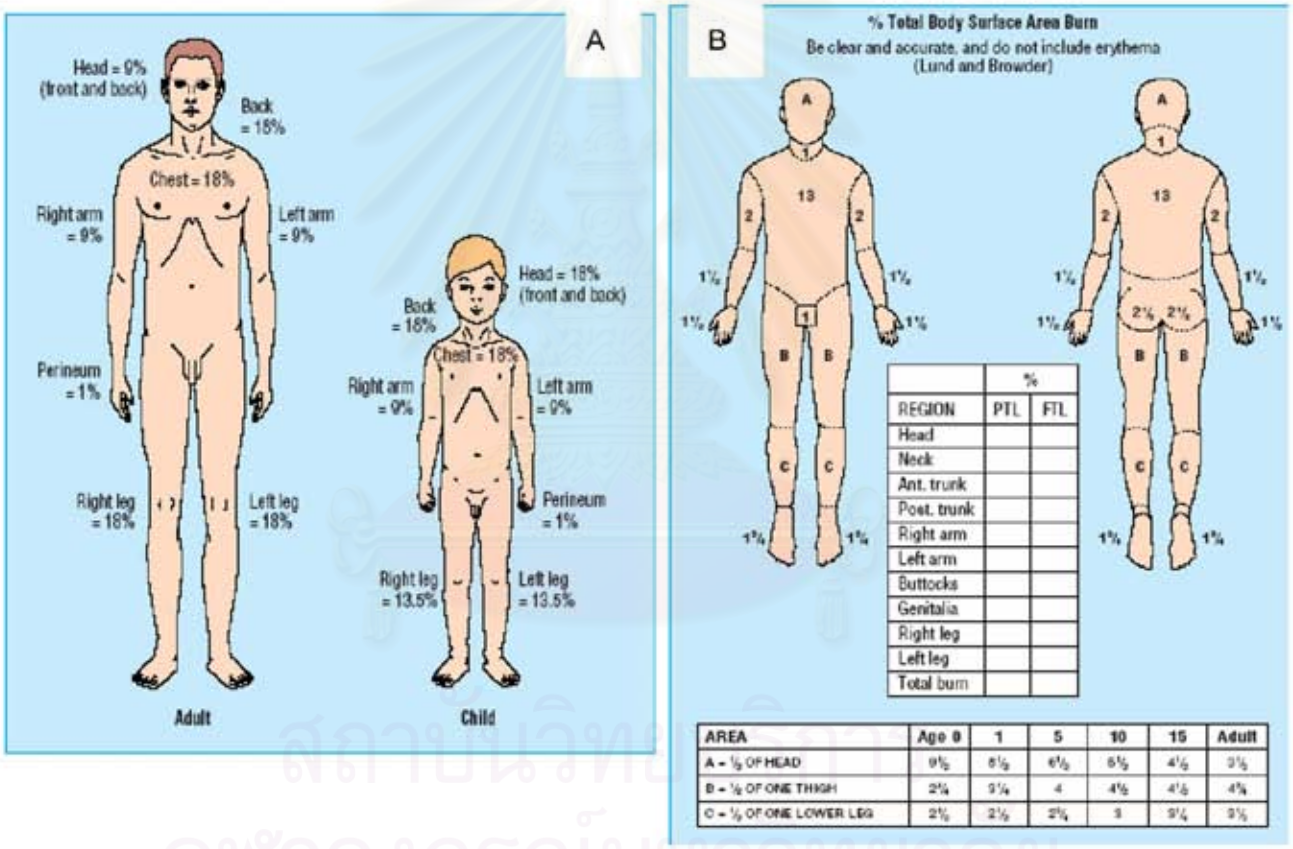


Figure 2.6 A: Wallace rule of 9s to estimate extent of burn injury by percentage
B: Lund and Browder chart is useful in assessing the extent of burn injury (the relative proportions of body areas differ in children) (Hettiaratch and Papini, 2004)

2.3.6 Burn wound healing

The burn wound is thought as the pivotal factor mediating many local and systemic disturbances that characterize burn injury. These include fluid and protein loss, local and systemic sepsis, gross metabolic, endocrine, haematological and immune disturbances (Dziewulski, 1992). The initial local effect of a burn is that of tissue damage and destruction. Regeneration is a feature seen in superficial partial-thickness burns. The injury involves the loss of epidermis and basement membrane and the papillary dermis. There may be a highly exudative and painful wound.

Re-epithelialization begins not just at the wound margin but also from the appendageal structures. Typically inflammation initiates a cascade of events with polymorphonuclear leukocytes begin attracted to wound site. Their principle role is proteolysis and phagocytosis of debris. They signal to macrophages which, when activated, enter the wound site to undertake a more detailed assessment of the damage and through a cytokine-mediated signaling process will recruit fibroblasts to begin the process of replacing the damaged collagen.

Granulation tissue consisting of new fibroblasts and endothelial tissue develops as the result of an inflammatory response at the margin of the wound (both the edges and the undersurface of the coagulated eschar). The eschar becomes loosened and will eventually slough. Because of the lack of skin appendages, the wound will help by contraction and epithelial growth from the edges. For most wounds of any size, the result of such natural wound healing is a contracture deformity and unstable scar. The end result of repair is the deposition of disorganized collagen, which is physically apparent as scar tissue. Scarring is the major cause of long-term morbidity after a burn and can result in physical disability when function is impaired and physiologic and social isolation

when deformity distorts features particularly on face and other regions of high esthetic impact (Latha and Babu, 2001; Burd and Chiu, 2005).

2.3.7 Free radical and antioxidant therapy

Burn wound healing is a normal reaction to injury and the formation of scar tissue occurs through a series of cellular and biochemical processes. The free radicals are involved in major physiological mechanisms such as phagocytosis, the inflammatory reaction and the reperfusion ischemia phenomenon observed during organ storage. During cutaneous thermal injury, several factors contribute to further tissue damage and important of these are the oxygen free radicals. Oxidative stress contributes to secondary tissue damage and impairs immune functions in patients after burn injury and finally leads to peroxidation (Rock *et al.*, 1997). Recent studies demonstrate that there is a close relationship between a lipid peroxide reaction and secondary pathological changes following burns (Saez *et al.*, 1984; Till, Hatherill and Toutellotte, 1985; Cetinkale *et al*, 1997). The study on lipid peroxidation product, the malonodialdehyde levels by the method of Yagi (Yagi, 1984) will give us the extent of free radical damage in the cells (Cetinkale *et al*, 1997).

Increased intracellular generation of free radicals has been implicated in: hyperoxygenation syndromes such as hyperbaric oxygen toxicity from respiratory dependency, ischemia reperfusion syndrome, ageing, drug-induced hemolytic anemias and vitamin E and vitamin A deficiency.

The oxygen derived radicals cause cellular injury by: degrading hyaluronic acid and collagen, destroying cell membranes through the peroxidation of fatty acids within the phospholipid membrane, disrupting organelle membranes such as those surrounding lysosomes and mitochondria

(Delmaestro et al, 1980) and interfering with important protein enzyme systems (e.g. Na^{+}/K^{+} ATPase, Ca^{++}/ATPase, glutamine synttase) (Weiss, 1986).

At this point the early intervention of antioxidant therapy will significantly help to restore cell mediated immunity; decrease free radical mediated damage and minimizes tissue destruction during extensive burn injury.

2.3.8 Management of burn injuries (Murkhtar and Jones, 2003; Papini, 2004)

The goals of burns treatment can be stated as survival of the patient with rapid healing of wound and epithelialization, with minimal scarring and abnormal pigmentation (Burd and Chiu, 2005). The most important consideration is that after a burn the protective function (Archer, 1998) of the skin are lost and burn wound needs immediate and appropriate topical treatment. A two major options in treating the burn wound with biologic (such as xenogenic, allograft) or nonbiologic dressings (such as antibacterial, occlusive, non occlusive, Figure 2.7) (Pruitt, 1997).

Optimum treatment of the wound reduces morbidity and, in larger injuries, mortality. It also shortens the time for healing and returns to normal function and reduces the need for secondary reconstruction (Papini, 2004).

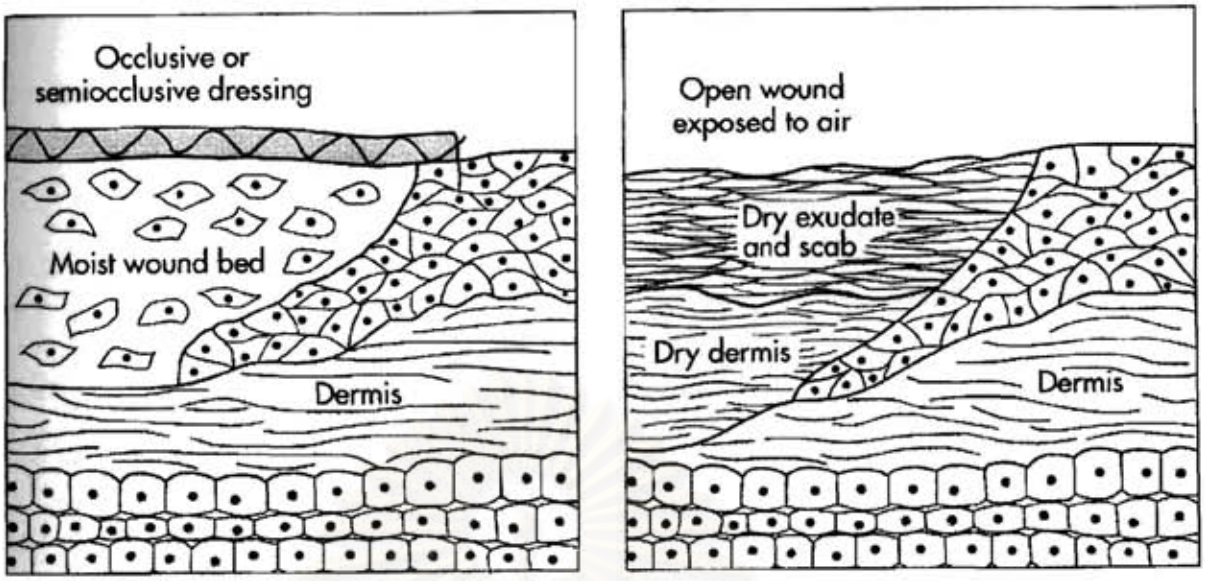


Figure 2.7 Treatment of wound (A) Occlusive dressing (closed method)
(B) Exposure therapy (open method)

2.4 Diabetes Mellitus and wound healing

DM one of the most important world health problems, is characterized by alterations in carbohydrate, fat and protein metabolism it is best characterized as a state of chronic hyperglycemia (World health Organization 1999). Fundamental to all types of diabetes is impairment of insulin secretion by the pancreatic beta cells. Chemical substances e.g. streptozotocin (STZ), alloxan and vacor, pancreatitis, or surgical pancreatectomy can damage beta cells.

In 1995, an International Expert Committee of the American Diabetes Association proposed a classification system that can be divided into five groups as follow:

1. Insulin dependent diabetes mellitus; IDDM or type I diabetes

Type1 DM, childhood diabetes, is characterized by loss of the insulin-producing beta cell of islets of Langerhans of the pancreas leading to a deficiency of insulin.

2. Non-insulin dependent diabetes mellitus; NIDDM or type II diabetes

Type2 DM, previously known as adult-onset diabetes, is due to a combination of defective insulin secretion and insulin resistance or reduced insulin sensitivity (defective responsiveness of tissues to insulin), which almost certainly involves the insulin receptor in cell membranes.

3. Gestational diabetes mellitus; GDM

Gestational diabetes also involves a combination of inadequate insulin secretion and responsiveness, resembling type 2 diabetes in several respects.

4. Impaired glucose tolerance; IGI and impaired fasting glucose; IFG

4. Other specific types of diabetes

Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis or hyperosmolar non-ketotic state) may occur if the disease is not adequately controlled. Serious long-term complications include microvascular damage, which may cause erectile dysfunction (impotence) and poor healing. Poor healing wounds, particularly of the feet, may lead to gangrene which can require amputation. There is considerable evidence that hyperglycemia causes many of the major complications of diabetes including retinopathy, neuropathy and macro- and

microvascular damage (The Diabetes Control and Complication Trial Research Group, 1993; De Fronzo, 1997; UK prospective Diabetes Study Group, 1998; Turner *et al*, 1998; Brownless, 2001). The pathogenesis of diabetic infection is multifactorial. The predisposing factors include hyperglycemia, impaired microcirculation, loss of sensation (Cavanagh, 1998) and suppressed cell mediated immunity (Hill et al, 1982).

Oxidative stress resulting from increased production of ROS plays a key role in the pathogenesis of late diabetic complication (King and Brownlee, 1996; Baynes and Thorpe, 1996; Sundaram, 1990; Nourooz-Zadeh *et al*., 1997; West, 2000; Brownless, 2001). Hyperglycemia-induced oxidative stress ultimately leads to tissue damage (King and Brownlee, 1996; Koya and King, 1998; Nishikawa, Edelstein and Rrownke, 2000; Nadler and Natarajan, 2000; Brownlee, 2001). Furthermore, ROS in the event following skin injury and, is known to impair healing process in human (Goodson and Hunt, 1979; Shula, Rasik and Patnaik, 1997; Sen et al, 2002) and delayed in the diabetic condition (Mastsuda, 1998).

The ability of antioxidants to protect against the effects of hyperglycemia and free fatty acids (FFA) in vitro, along with the clinical benefits often reported following antioxidant therapy, support a causative role of oxidative stress in mediating and/or worsening these abnormalities (Evans, 2002). It significantly prevents tissue damage and stimulates the wound healing process. Many plant extracts and medicinal herbs have been shown potent to process antioxidant and antidiabetic activity (Tapiero, 2002; Larkins, Nicholas and Wynn, 2004; Chanwitheesuk, 2005; Rahimi *et al*., 2005). Natural antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots flowers, pollen and seeds) (Chanwitheesuk, 2005).

Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of skin is decreased (Spanheimer, Umpierrez and Stumpf, 1988), as a result of reduced biosynthesis and/or accelerated degradation of newly synthesized collagen (Chithra, Sajithal and Chandrakasan, 1998). Deep skin wounds in non-diabetes were healed by contraction

and granulation tissue formation. In contrast, diabetic wound closure is predominantly due to the formation of granulation tissue and re-epithelialization (Albertson *et al.*, 1993). However, the molecular mechanisms that delay the healing process are poorly understood.

2.5 Wound healing in rat models

One of advantages of using animals is that the wound healing process is so accelerated in animals so that it is possible to study the process over days rather than over the weeks required in humans (Cross et al, 1995). However, rat skin wound healing dose not perfectly mimic human skin wound healing because the skin morphology is different. Rats are described as loose-skinned animals whereas human have tight skin(Marx and Mou, 2002). Rats possess a subcutaneous panniculus carnosus muscle, which human lack. This muscle contributes to skin healing by both contraction and collagen formation (Gottrup et al, 2000). Rat are not subject to scurvy and therefore do not require the addition of vitamin C, which is essential for collagen synthesis, to their diet. Rats possess the enzyme L-gluconolactone that converts L-gluconogammalactone to vitamin C, whereas primates and guinea pigs lack this enzyme. However, skin of rats has epidermis, basement membrane and dermis similar to skin of human. Although there are inherent drawbacks in using rats for comparisons with human skin wound healing, there are also advantages in the use of rats as a research model.

The rat has cutaneous microvascular flow properties affording an excellent comparative model for human skin blood (Rendell *et al*, 1993). Previous studies demonstrated that the rat provides an effective model for skin blood flow change in man. Several studies have used the rat model to study wound healing and bum injury. Similar to human, it has been shown that alterations of malondialdehyde and glutathione levels, myeloperoxidase activity and collagen content were observed in burn trauma of rats.

STZ-induced DM is associated with rapid and progressive alteration in capillary permeability in the peripheral vasculature of the skin. These findings indicate that diabetic vasculopathy, characterized in part by enhanced capillary permeability, result from abnormal activation of several and different pathophysiological pathways. Sibi *et al.*, (2005) investigated the effect of STZ-induced type 1 diabetes is associated with an increase in vascular permeability in skin. This elevated vascular permeability was detected 1 week following the induction of diabetes and persisted over 4 weeks. Such findings are important to better define the physiological mechanisms involved in long-term development of diabetic complications.

2.6 Malvastrum coromandelianum (L.) Garcke

MC is a perennial or annual (sometime) herb belonging to family Malvaceae. It is locally known as Dai-Kat or Ya-Tevada. It is broad-leaved, erect herbs or-shrubs (sub-shrub), up to 0.5 (-1.5) m high. Flowers are solitary, axillaries, predominantly yellow, pedicellate. Fruit is schizocarpic (Figure 2.8).

Figure 2.8 *Malvastrum coromandelianum* (L.) Garcke

2.6.1 Pharmacological study

MC or Dai-Kat in Thai was one of medical plants scientifically investigated by The Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Health of Thailand. A wide spectrum of pharmacological activity has been reported as follows.

2.6.1.1 Hypoglycemic activity

MC extract has been claimed to treat DM. Attawish *et al.* (1999) previously showed that water extract of this medicinal plant exhibited hypoglycemic effect in diabetic rabbits. In 2004, Rattanajarasroj et al investigated the hypoglycemic activity of MC-1 extract and its separated fractions which were given orally at doses of 1, 2, 4 g/kg B.W to normal and alloxan-induced diabetic rats. The result demonstrated that while all separated fractions did not show any hypoglycemia activity, MC-1 extract (a portion of water extract) exhibited the hypoglycemic activity which might be attributed to various compounds in MC-1 extract.

Jesadanont et al. (2005) demonstrated that water extract from whole plant of MC exhibited strong hypoglycemic activity when being administered orally. Feeding the spray-dried crude water extract as low as 50 and 100 mg per kg body weight to streptozotocin-induced male Wistar rats, could reduced blood glucose. The result showed hypoglycemic effect of MC extract. The same results was also reported by other investigators (Pongpech *et al.* 2005)

2.6.1.2 Antibacterial activity

Water extract from whole plant of MC showed *in vitro* inhibitory effect against *Staphylococcus aureus* at 25% MC extract (Pongpech *et al.* 2005).

2.6.1.3 Antipyretic, analgesic and anti-inflammatory effects

Antipyretic, analgesic and anti-inflammatory effects of MC extract were tested by fever induced by typhoid-paratyphoid vaccine in rabbits. In addition hot-plate test, writhing test, ear edema, and abdominal capacity permeability of mice were also used to assess the activities. Result of study showed that both aqueous extract of whole plant or root of MC at dose of 10, 20 mg/kg, equivalent to the crude plant could lower rabbit fever, decrease mice writhing, inhibit ear edema and decrease the permeability of the blood capillary in abdominal cavity of mice (Moulun *et al*,1999)

2.6.2 Chronic toxicity study

Attawish *et al.* (1998) reported the chronic toxicity study of this plant in Wistar rats. Water extract of MC at the dose equivalent to crude drug of 0.2, 2 and 20 g/kg BW/day was given orally to animal for six-month period. It was found that body weight gain and hematological parameters of all extract-treated groups were not significantly different from those of the control group; Histopathological study of internal organs did not reveal any abnormalities that could be attributed to the toxicity of extract.

CHAPTER III

METERIALS AND METHODS

3.1 Materials

3.1.1 Chemical substances

- Streptozotocin (STZ, Sigma, USA)
- Citrate buffer (Sigma, USA)
- Hematoxylin (Bio-optica, Italia)
- Eosin (Bio-optica, Italia)
- Alcohol (Siribuncha Co.,Ltd, Thailand)
- Formaldehyde (FORMALIN®, Vidhyasom Co.,Ltd, Thailand)
- Xylene (TJ Baker, USA)
- Paraffin (Tyco Healthchare group LP, USA)
- Chloral hydrate (Asia Pacific Specially Limited, Australia)
- Sodium pentobarbital (NEMBUTAL®, Sanofi, France)
- Normal saline solution (Klean & Kare, Thailand)
- Thrichloroacetic acid (Sigma, USA)
- Butylatehydroxy toluene (Sigma, USA)
- Methanol (Lab-scan Asia, Thailand)
- Thiobarbituric acid (Sigma, USA)

3.1.2 Instrument

- Electrical hot plate
- Laser Doppler flowmeter
  (MoorLab-ServerS/N FEO241, Moor Instruments, England)
- Battalion
- Glucometer (Accu-Chek Advantage, USA)

- Glucose oxidase reagent strips (Accu-Chek Advantage, USA)
- Cover glass
- Slide
- Rotary microtome (Leica, Vashaw Scientific, Norcross, GA)
- Image Tool V.30
- Color photograph
- Others: Syringes, Needles, Cotton pads

3.1.3 Experimental animal

Male Wistar rats weighing 250-300 gm purchased from The National Laboratory Animal Center, Salaya, Mahidol University, Bangkok were used in this study. The rats were caged (five rats per cage) in the air-conditioned room maintained temperature at 25±1°C. They were fed with commercial pellet diet CP mice fed, Pokphand Animal Fed Co, Ltd. Bangkok, Thailand. They were provided with food and water *ad libitum*. The rats were used after acclimatization to the laboratory environment for a 7-day period.

All animal care and handling were conducted with the approval of the Ethical Committee of the Faculty of pharmaceutical Sciences, Chulalongkorn University.

3.2 Method

3.2.1 Diabetic induction

To induce diabetes mellitus, STZ solution was freshly prepared by dissolving STZ in citrate buffer pH 4.5 and immediately injected into the tail vein, at dose of 60 mg/kg BW. Blood glucose was determined by using glucometer. Samples were analyzed by applying a drop of blood to a prepared strip. Rats treated with STZ that did not exhibit an elevation of blood glucose level greater than 200 mg/ml at 7 days were excluded from the study (Chithra, 1998).

3.2.2 Preparation of aqueous extract of *Malvastrum coromandelianum*

Leaves and stem of MC dried at 60°C, pulverized and then extracted three times by distilled water. First extractions were made by 80°C distilled water with a ratio of 100 l/10 kg. Dry weight for 7-8 hours and then filtered.

The concentration of MC water extract used in this study was 1, 5 and 10 w/v in distilled water in freshly prepared solution. Initially, 0.01, 0.05 and 0.1 gm of the extract were dissolved in 9 ml of distilled water. Volume was adjusted for obtain 1%, 5% and 10% (w/v) of the extract of using to be used

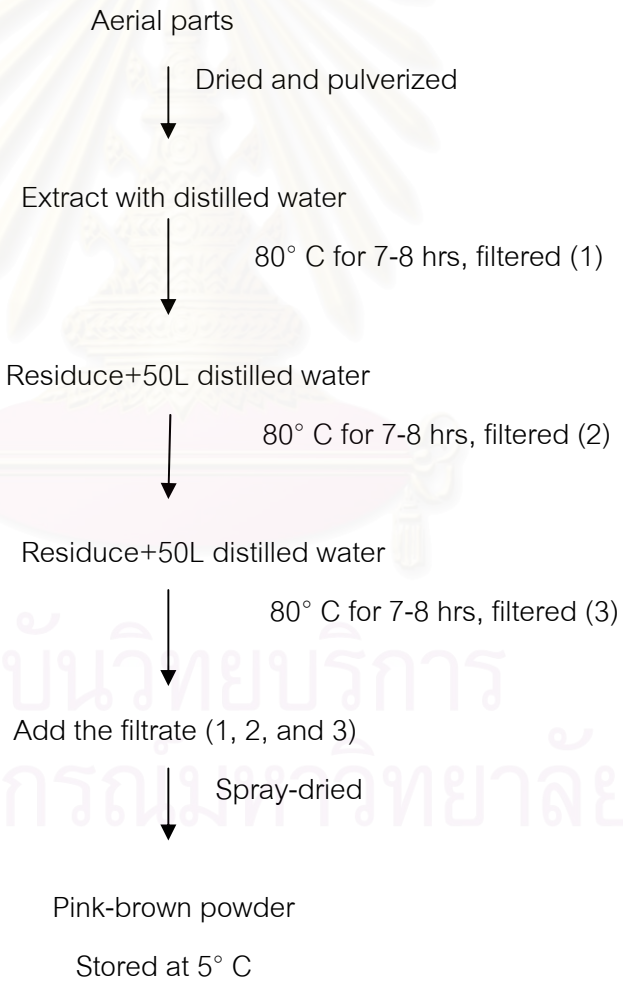


Figure 3.1 Extraction procedure

3.2.3 Microbial limit test

The microbial limit tests are designed to perform the qualitative and quantitative estimations of specific viable microorganisms present in MC samples before applying to the animals. Samples are mixed in sterile distilled water and used as the test fluid. The fluid samples are streaked into a sterile agar sample (Mueller-Hinton Agar) in a aseptic petri dish and incubated at 37°C for 24 hours. The numbers of microorganism of samples are counted.

3.2.4 Induction of second degree of burn injury

The effect of MC extract on burn was investigated using the method of Soomboonwong et al. (2002) which was modified from Zawacki (1974). The animals were anesthetized with sodium pentobarbital 60 mg/kg B.W., intraperitoneally. Back of animals between lower parts of scapulas were shaved and depilated. Second-degree skin burn was made by placing the 90°C hot plate (diameter 2 cm) (Figure 3.2) on the selected area of the back for 10 s (Sener et al., 2002; Cakir et al., 2004). The wounded area of each animal was measured immediately after burning on day 3, 7 and 14 posts burning (Figure 3.3). It involves about 12.5 cm, approximately 2.5% of the total body surface area of animal. The color photograph of the wounds was taken by using digital camera and areas of wound were measured by using Image Tool v.30. The degree of wound healing was calculated by the method as described by Reddy et al. (2002). The animals were housed and were fed with commercial pellet and free access of water for 3, 7 and 14 days in which the wound were treated daily with one of the test substances (1ml) topically. On days 3, 7 and 14 posts burning, the animals were sacrificed with chloral hydrate 100 mg/kg B.W., intraperitoneally. One-half of the tissue samples in the healed wound were isolated from each animal for histological examination and another half was used for lipid peroxidation assay.

Figure 3.2 Electrical hot plate

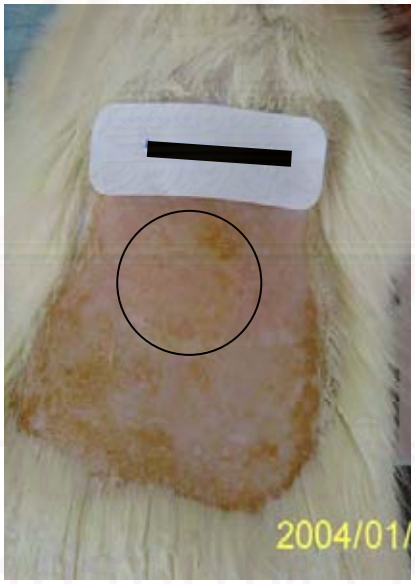


Figure 3.3 The area prepared for wounding of burn wound. The burned area of animal was measured immediately after burning. The color photograph of the wounds were taken by using digital camera, the wound area was indicated by circle line (Bar = 2 cm)

3. 2.5Animal preparation

A total of 180 male Wistar rats were divided into two groups of 90 animals each for non-diabetic rats and diabetic rat. In each group, the animals were subdivided into six subgroups of 18 animals each.

Non-diabetic group: The animals were divided randomly into five groups as follows:

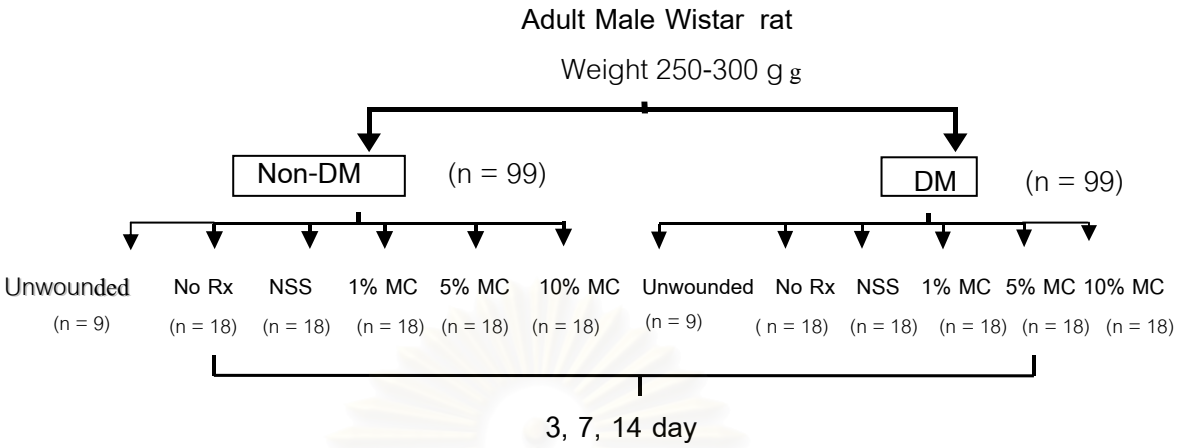
1. Non-diabetic rat burn without treatment
2. Non-diabetic rat burn treated with normal saline solution (NSS)
3. Non-diabetic rat burn treated with 1% MC extract
4. Non-diabetic rat burn treated with 5% MC extract
5. non-diabetic rat burn treated with 10% MC extract

Diabetes group: The animals were divided randomly into five groups as follows:

1. DM rat burn without treatment
2. DM rat burn treated with normal saline solution (NSS)
3. DM rat burn treated with 1%MC extract
4. DM rat burn treat with 5%MC extract
5. DM rat burn treat with 10%MC extract

In addition 18 animals (9 in diabetics and 9 in non-diabetics) without wound were used as references in measurement of blood flow and MDA.

In day 3, 7, 14, six animals from each group were randomly taken for the evaluation of the wound.



1. Degree of wound healing: Size of wound
2. Cutaneous blood flow: Laser Doppler Flowmeter
3. Antioxidant activity: Lipid peroxidation assay (MDA)
4. Histological study: Hematoxylin and Eosin dyes

Figure 3.4 Diagram of experimental animal group

3.3 Evaluation of burn wound

3.3.1General appearance of the wound

The lesion of wounds was grossly examined on day 3, 7, and 14 posts burning. The wounds were examined in terms of color, exudates, swelling of wound surface and the consistency of surrounding wound tissue.

3.3.2Wound healing

On day 3, 7 and 14 posts burning, the color photographs of the wounds were taken by digital camera. The areas of wound were measured by Image Tool v.30 and the degree of wound healing was calculated using the following formula (Reddy et al, 2002)

$$\text{The degree of wound healing (\%)} = \left[1 - \frac{\text{wound area on corresponding day (cm}^2\text{)}}{\text{wound area on zero day (cm}^2\text{)}}\right] \times 100$$

3.3.3Cutaneous blood flow

Measurement of cutaneous blood flow using Laser Doppler Flowmeter (LDF, Figure 3.5) was performed according to Lindbloma et al. 2000 (Figure 3.5). The method, modified from Eun (1995) and Fagrell (1995), was used in this study. The needle probe was fixed perpendicularly to and above the skin about 1 mm. Five different measurements (at the center and four corners) were performed at each time and the mean value was used for calculation of the percent change compare with the normal untreated rats (Figure 3.6).

Figure 3.5 Laser Doppler flowmeter

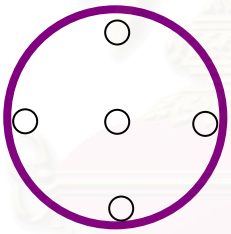


Figure 3.6 Selected position in the wound area for the measurement of blood flow

3.3.4Lipid peroxidation assay

Lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) which is an end product of lipid peroxidation using the method described by Madreson, 1985 and Wei et al. 2002. Sample of skin weighing 0.17-0.25 g were homogenized in 1.8 ml of 10% trichloracetic acid and 0.2 ml of butylatehydroxy toluene in methanol (0.5 g/L). The homogenate was heated at 100°C for 30 min and cooled down at the room temperature. After centrifugation at 3000 rmp, the resultant supernatant was mixed with the equal volume of 0.67% 2-thiobarbituric acid and incubated at 100°C for another 30 min and then centrifuged at 3000 rpm for 10 min. Concentration of MDA was calculated based on the absorbance determined by a spectrophotometer at 532 nm and the final result was expressed as nmol of MDA per g/tissue of skin.

3.3.5Histological analysis

The specimen of skin, 0.5 cm in size, was taken from half of the burn area. The tissue were preserved in the fresh fixative aqueous 10% neutral buffered formalin solution for at least 24 hrs and embedded in paraffin. The section of 20 μm in thickness were cut and stained with hematoxylin and eosin dyes. The light microscope (Nikon 516609) with x4 and x10 objective lens was used.

3.4Statistical analysis

Results are presented as mean $\pm$ S.E.M. The differences among experimental groups were compared by one-way ANOVA followed by Least significant different test (LSD) and were considered statistically when P was less than 0.05.

CHAPTER IV

RESULTS

4.1 The degree of wound healing

The degree of wound healing was calculated on day 3, 7 and 14 posts burning by using the formula previously described (Reddy, 2002).

4.1.1 Non-diabetic rats

4.1.1.1 Day 3 post burning

On day 3 post burning, the degree of wound healing of untreated (14.00 ± 1.91%) was not significantly different from NSS-treated group (20.5 ± 4.05%). In the extract-treated group, degree of wound healing in 1% (33.83 ± 3.34%) but not 5% (27.00 ± 2.16%) and 10% (20.33 ± 3.12%) of MC extract were significantly different from those found in NSS-treated group (Figure 4.1).

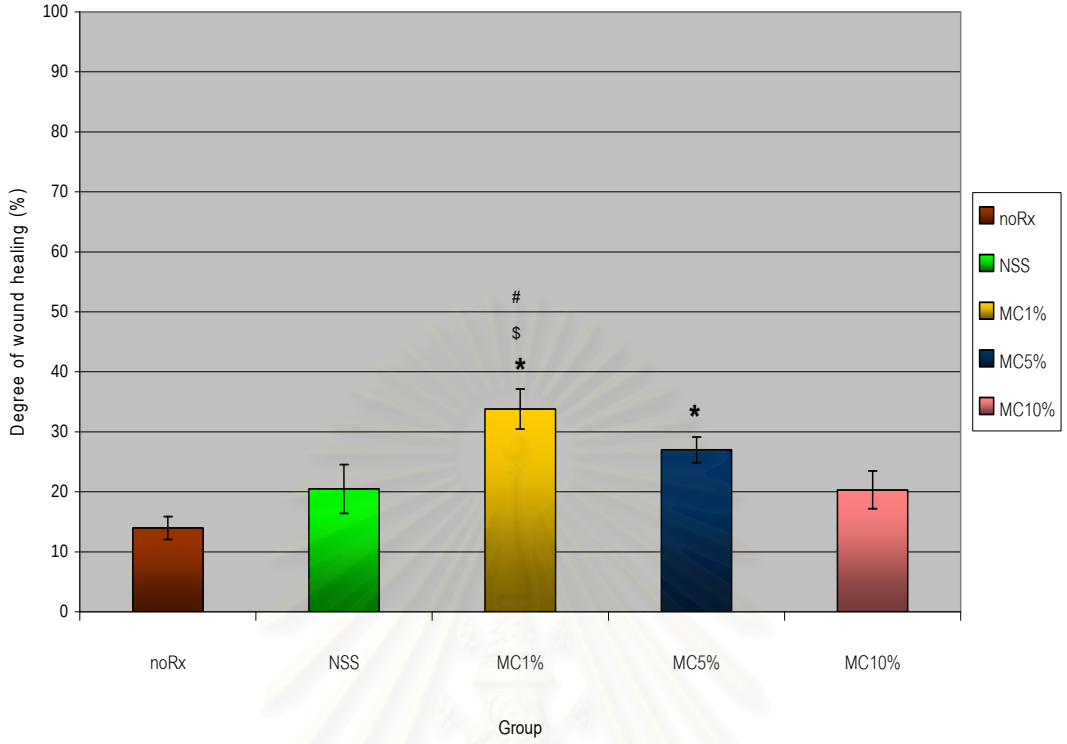


Figure 4.1 Degree of wound healing on day 3 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

Significant difference as compared to 10%MC group ($p<0.05$)

4.1.1.2 Day 7 posts burning

On day 7 posts burning, a similar profile of response, but with higher degree of wound healing was noted. The degree of wound healing of untreated (39.83 ± 3.34%) was not significantly different from NSS-treated group (43.16 ± 3.85%). Degree of wound healing in 1% (63.83 ± 4.14%) but not 5% (56.5 ± 5.31%) and 10% (48.00 ± 4.98%) of MC extract were significantly different from found those in NSS-treated (Figure 4.2).

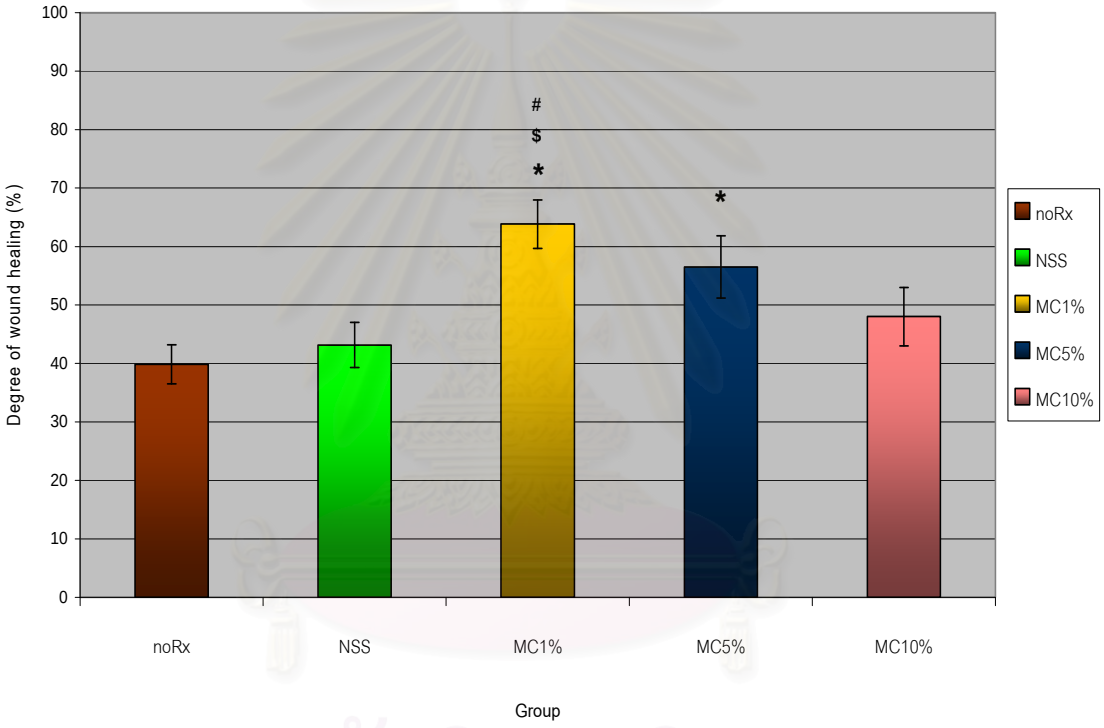


Figure 4.2 Degree of wound healing on day 7 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

Significant difference as compared to 10%MC group ($p<0.05$)

4.1.1.3 Day 14 posts burning

On day 14 posts burning, further increases degree of wound healing was noted in all experiment groups. In contrast to the healing on day 3 and 7, the wound healing effect of 10% MC was demonstrated. The degree of wound healing in untreated (73.50 ± 5.43%) was not significantly different from NSS-treated group (81.83 ± 3.36%). Similar the degree of wound healing (95.16 ± 2.50%, 96.83 ± 1.44% and 95.16 ± 3.52% were observed in 1%, 5% and 10% of MC extract-treated rats) were significantly different from NSS-treated group (figure 4.3).

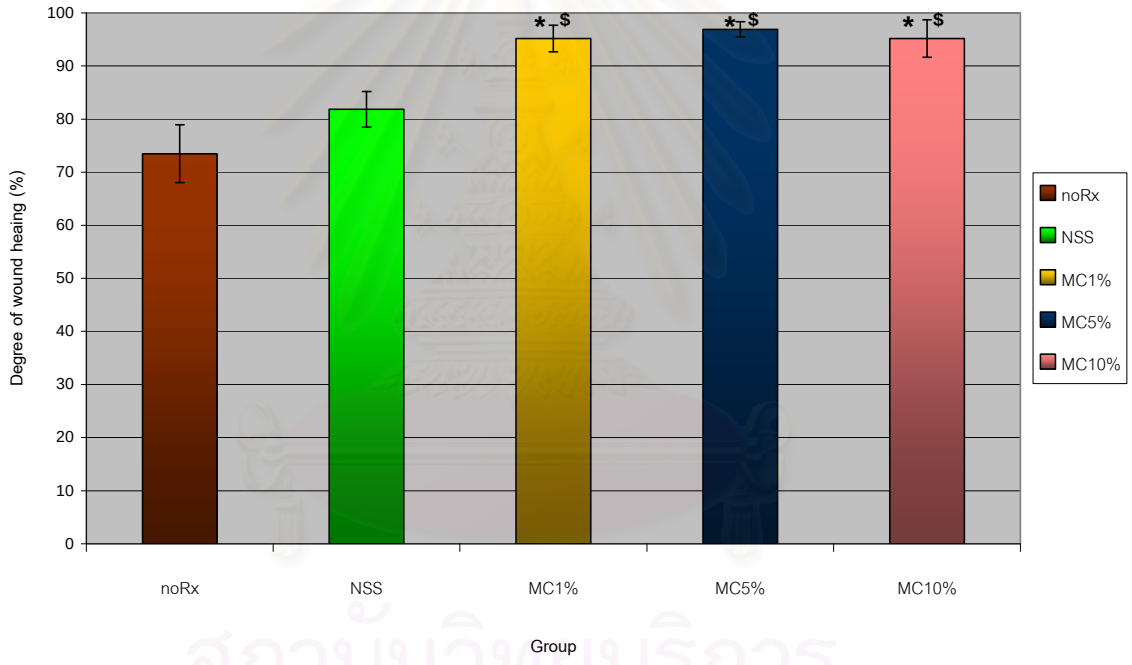


Figure 4.3 Degree of wound healing on day 14 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

\# Significant difference as compared to 10%MC group ($p<0.05$)

4.1.2 DM rats

4.1.2.1 Day 3 posts burning

On day 3 post burning in diabetes rats, the degree of wound healing in untreated (12.33 ± 2.18%) was not significantly different from NSS-treated group (15.83 ± 6.19%). Furthermore, the degree of wound healing in 1%MC (28.16 ± 3.84%) was significantly different from untreated group (Figure 4.4).

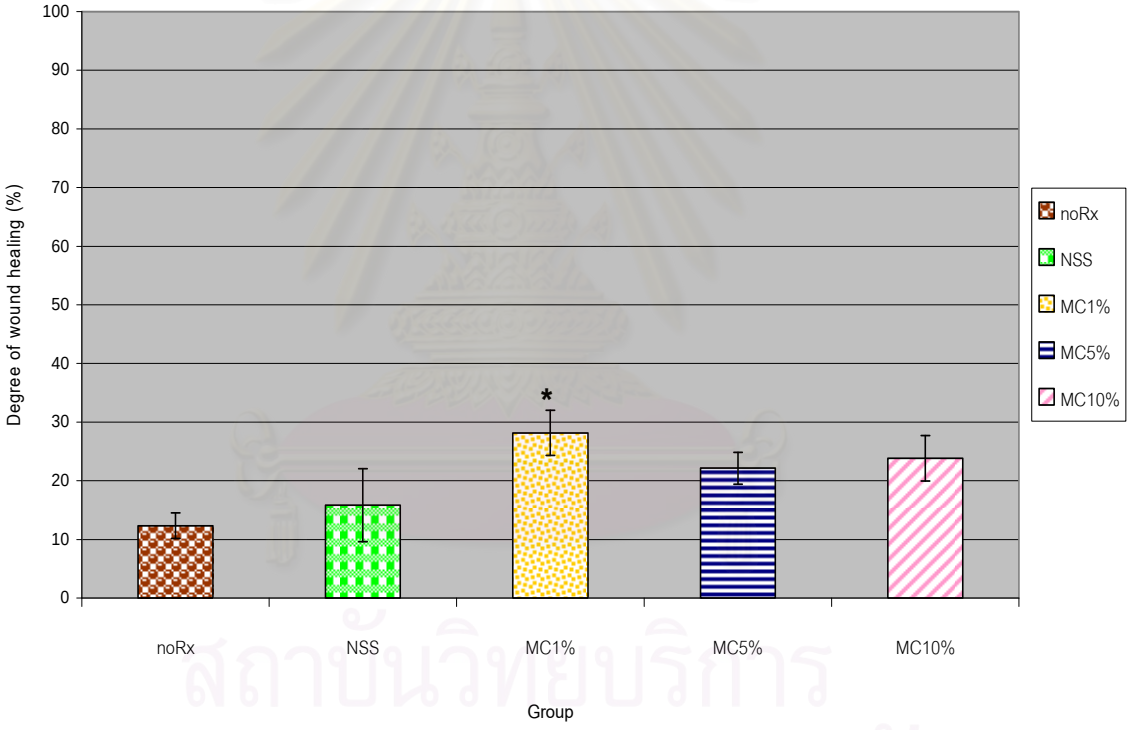


Figure 4.4 Degree of wound healing on day 3 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn. .

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

4.1.2.2 Day 7 post burning

On day 7 post burning in diabetes rats, The degree of wound healing in untreated (19.66 ± 1.47%) was not significantly different from NSS-treated group (20.83 ± 3.28%). In contrast, the degrees of wound healing in the animals from extract-treated group (37.66 ± 2.61%, 30.50 ± 4.19%, 31.00 ± 3.54% in 1%, 5% and 10%MC water extract respectively) were significantly different from untreated and NSS-treated group (Figure 4.5).

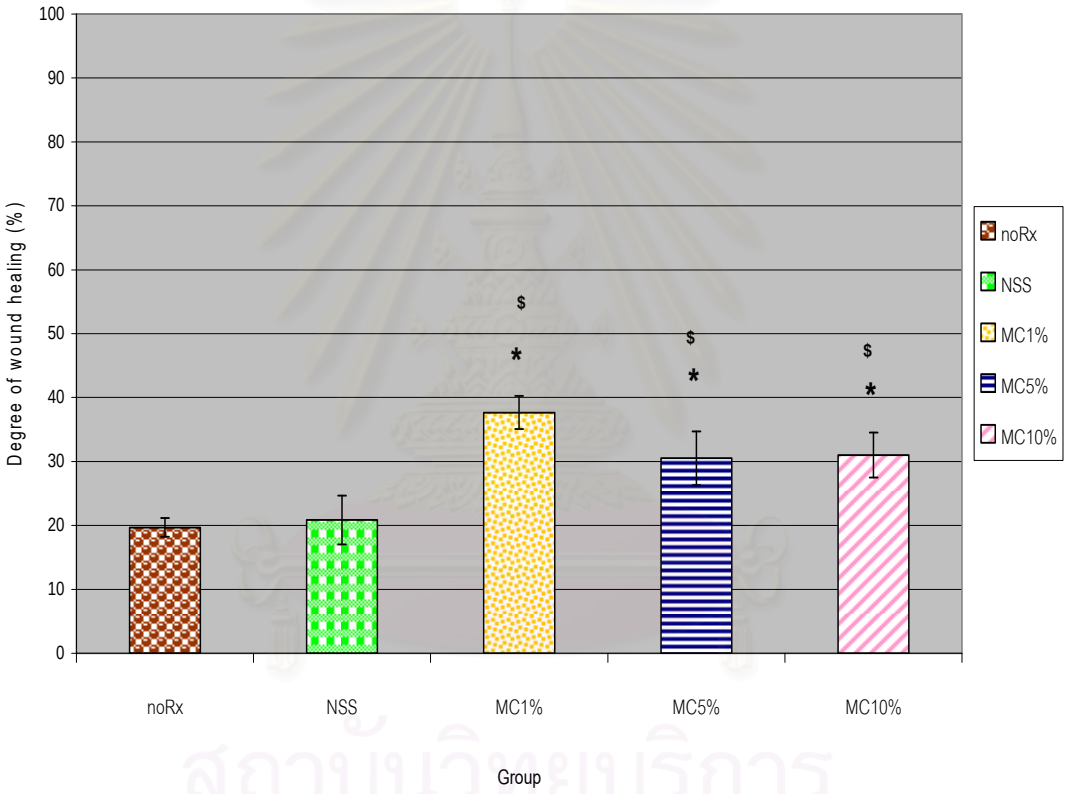


Figure 4.5 Degree of wound healing on day 7 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn. . .

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

4.1.2.3 Day 14 post burning

Similar results were observed on day 14 post burning in diabetes rats. The degree of wound healing in untreated animals (55.50 ± 3.97%) was not significantly different from NSS-treated group (57.83 ± 9.36%). Degree of wound healing in 1%MC, 5%MC and 10%MC were significantly different from untreated group and NSS-treated group (74.16±6.12%, 74.00±7.40%, 75.16±8.06% respectively). (Figure 4.6)

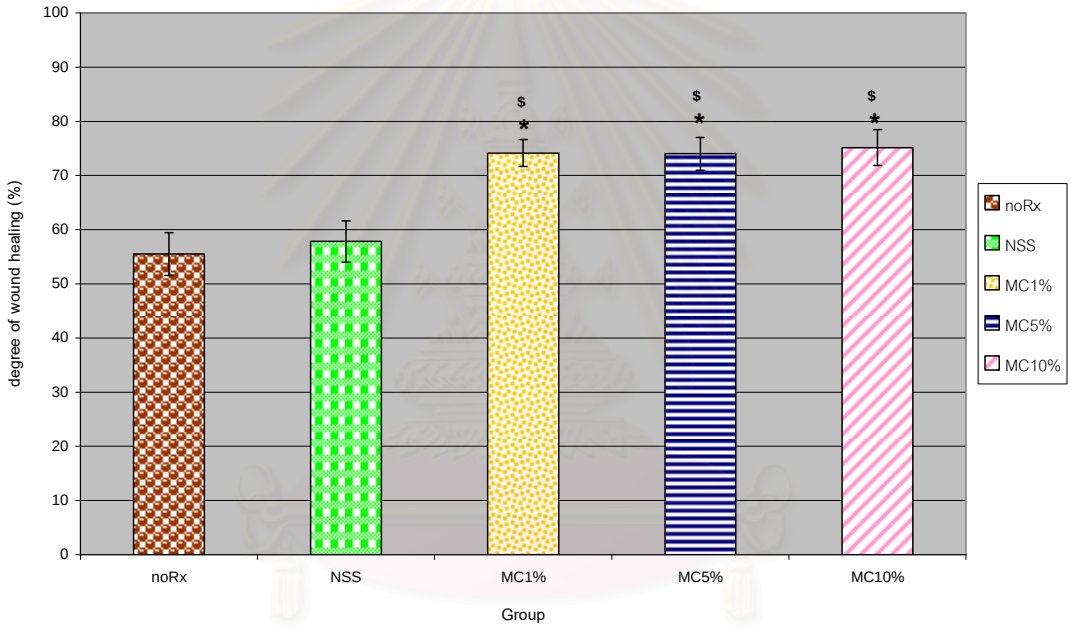


Figure 4.6 Degree of wound healing on day 14 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

4.2 Gross pathology evaluation

Gross pathological evaluation was made on day 3, 7 and 14 after burning by an observation of wound lesion and assessment of degree of wound healing.

4.2.1 General appearance of the wound in normal rats

4.2.1.1.1Non-diabetic rats

On day 3, the wound in untreated, NSS-treated group become swelling and exudates (Figure 4.7A, 4.7B). Comparatively, the wound in all MC extract treated group showed wound surface was rather dry (Figure 4.7C, 4.7D, 4.7E).

On day 7, the wound in untreated, NSS, 1%, 5%, and 10%MC extract showed red color, thickening of the wound size remain reduced from the first day. Most of wound treated with MC extract showed wound contraction, smaller in size compared the untreated and NSS-treated group (Figure 4.8 A-E).

On day 14, which was the end of experiment, untreated and NSS-treated group showed delay of epithelialization and the wound size were slightly decreased compared with those at the beginning. The wound in MC extract group showed complete of wound area (Figure 4.9 A-E).

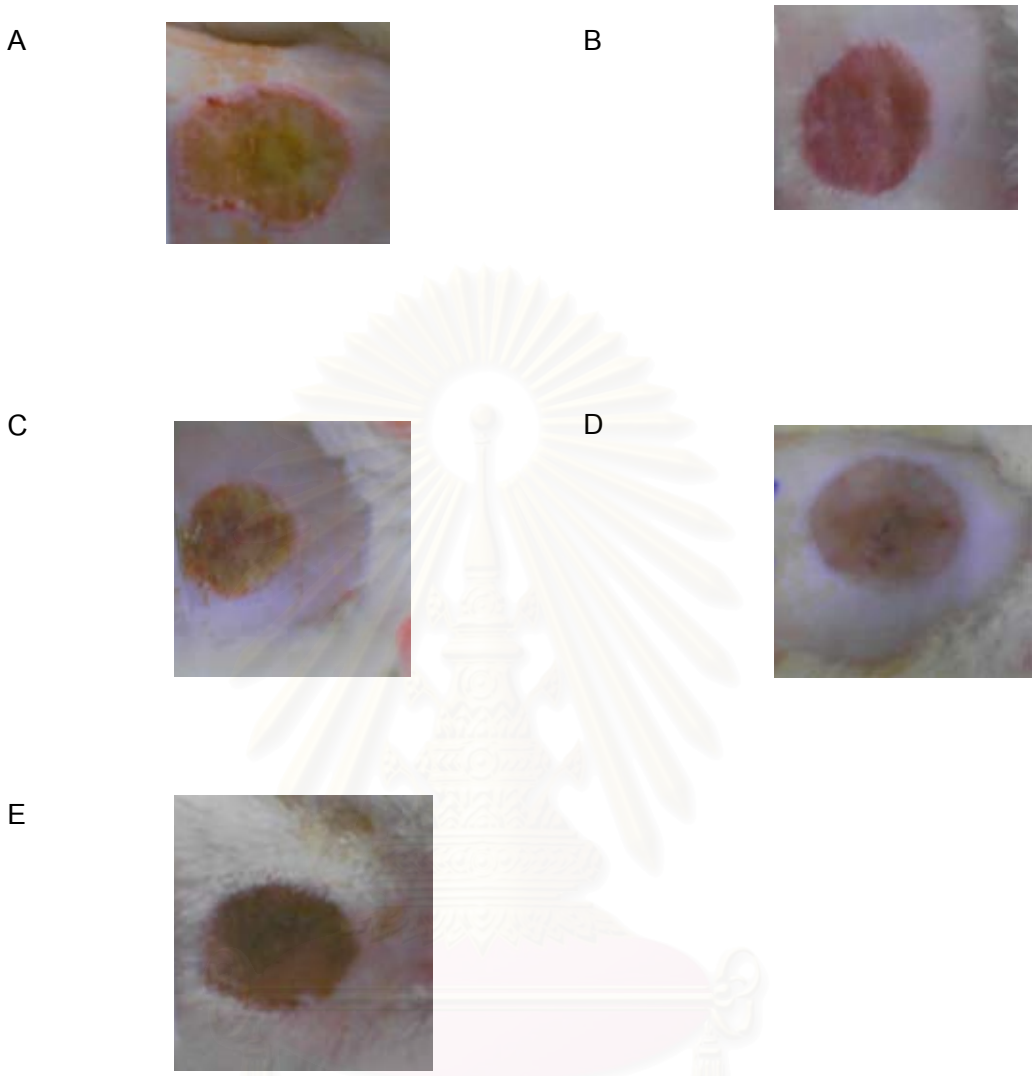


Figure 4.7 Burn wound on day 3 post burning in non-diabetic rats.

A) Untreated wound B) Non-DM Burn+NSS

C) Non-DM Burn+1%MC D) Non-DM Burn+5%MC

E) Non-DM Burn+10%MC

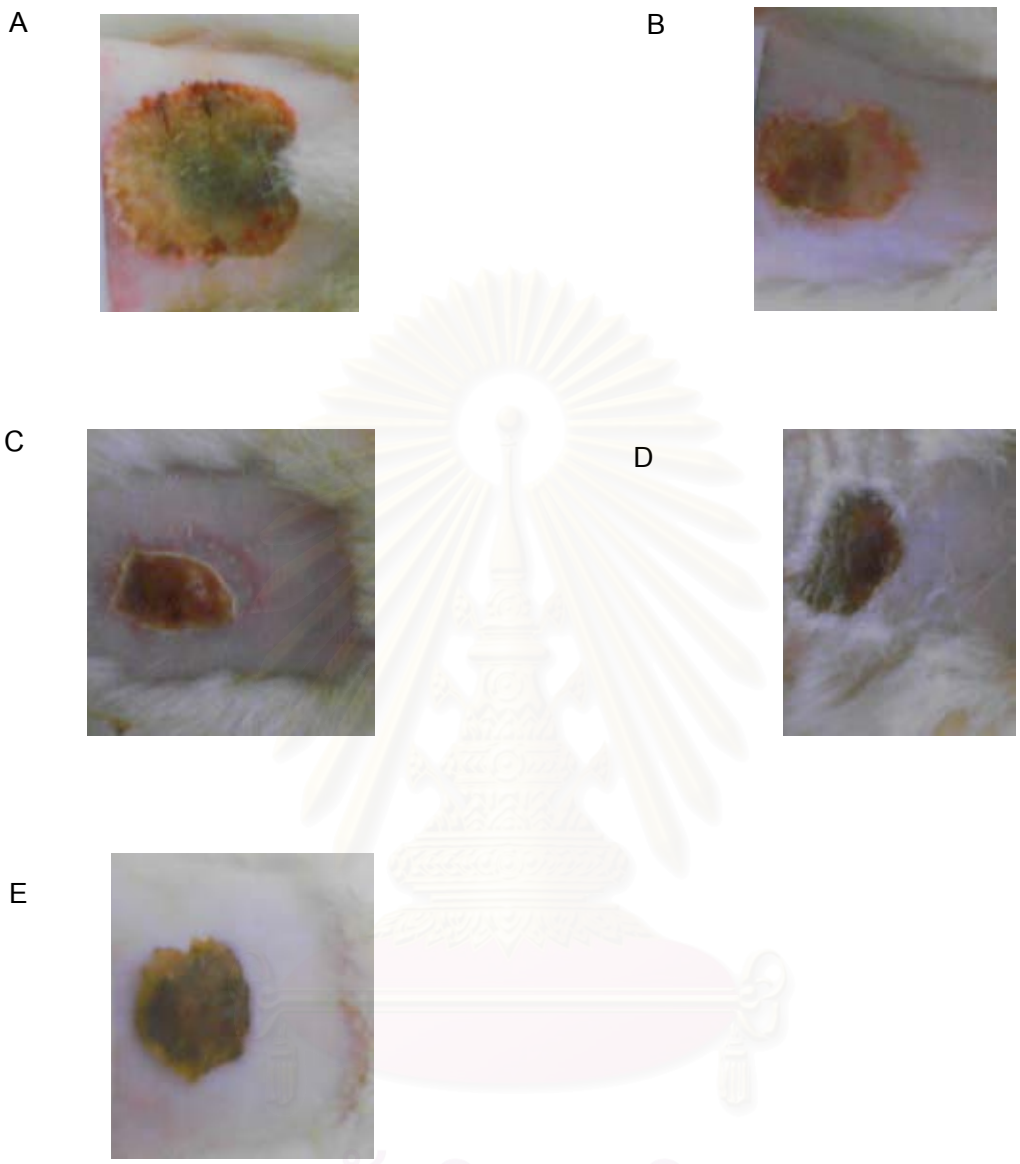


Figure 4.8 Burn wound on day 7 post burning in non-diabetic rats.

A) Untreated wound B) Non-DM Burn+NSS

C) Non-DM Burn+1%MC D) Non-DM Burn+5%MC

E) Non-DM Burn+10%MC

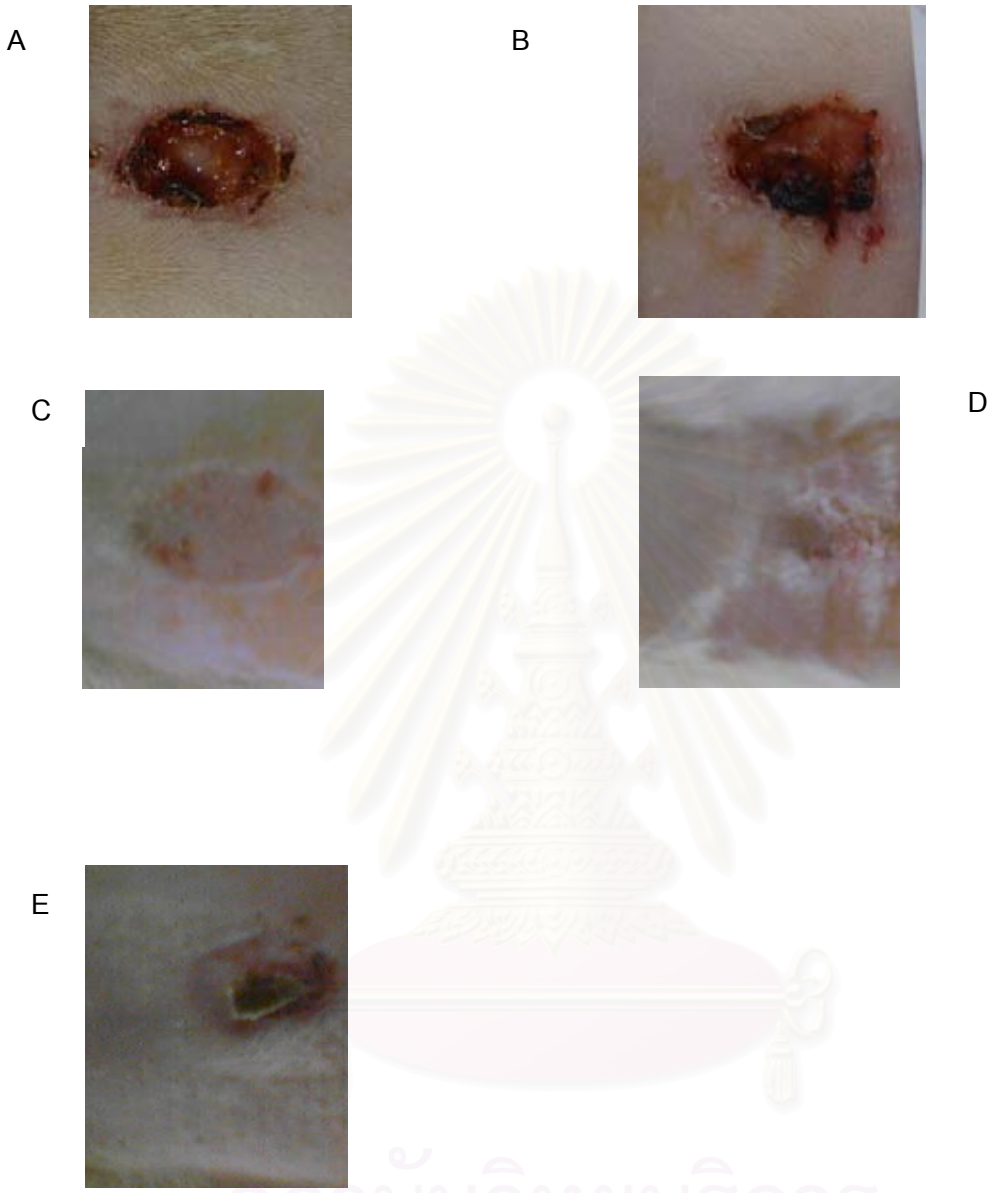


Figure 4.9 Burn wound on day 14 post burning in non-diabetic rats.

A) Untreated wound B) Non-DM Burn+NSS

C) Non-DM Burn+1%MC D) Non-DM Burn+5%MC

E) Non-DM Burn+10%MC

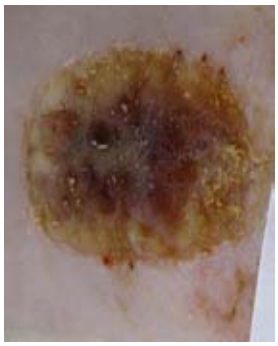
4.2.2 General appearance of the wound in diabetic rats

On day 3 post burning, wound in untreated and NSS-treated groups showed modulated exudation and swelling. All wound in MC extract treated group showed dry wound surface, progressive wound contraction and reduced of wound size (Figure 4.10 A-E).

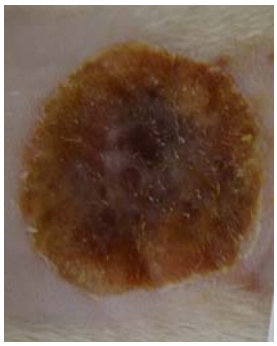
On day 7, wound in untreated and NSS-treated group showed swelling and red color, thickening of the skin at the wound and the wound size slightly decreased compared with those at beginning. MC extract group showed decreased in wound size (Figure 4.11 A-E).

On day 14, wound size in untreated and NSS-treated remains swelling and wound surface covered by scabs. MC extract group showed remarkable decrease in wound size and continuous growth hair at wound site (Figure 4.12 A-E).

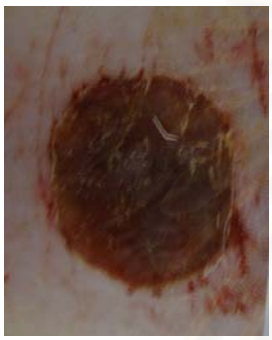
A



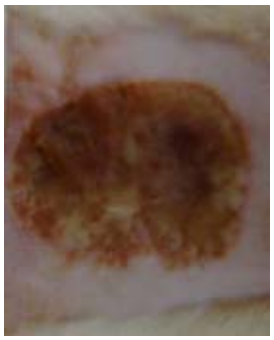
B



C



D



E

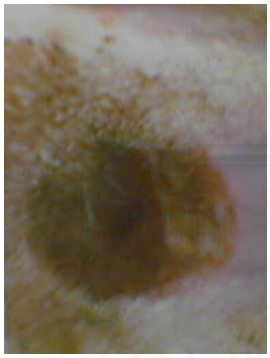


Figure 4.10 Burn wound on day 3 post burning in diabetic rats.

A) Untreated wound B) DM Burn+NSS

C) DM Burn+1%MC D) DM Burn+5%MC

E) DM Burn+10%MC

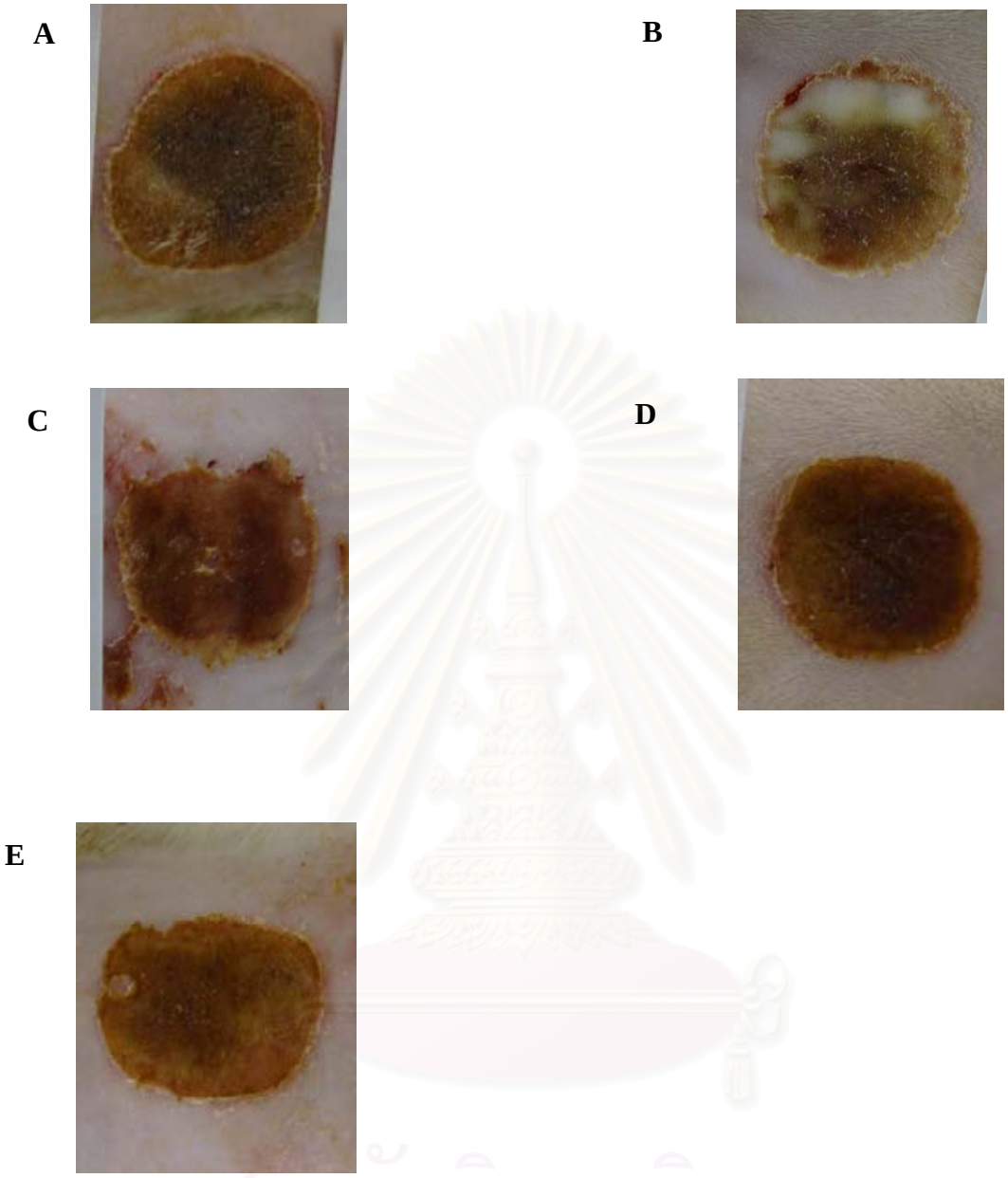


Figure 4.11 Burn wound on day 7 post burning in diabetic rats.

A) Untreated wound B) DM Burn+NSS

C) DM Burn+1%MC D) DM Burn+5%MC

E) DM Burn+10%MC

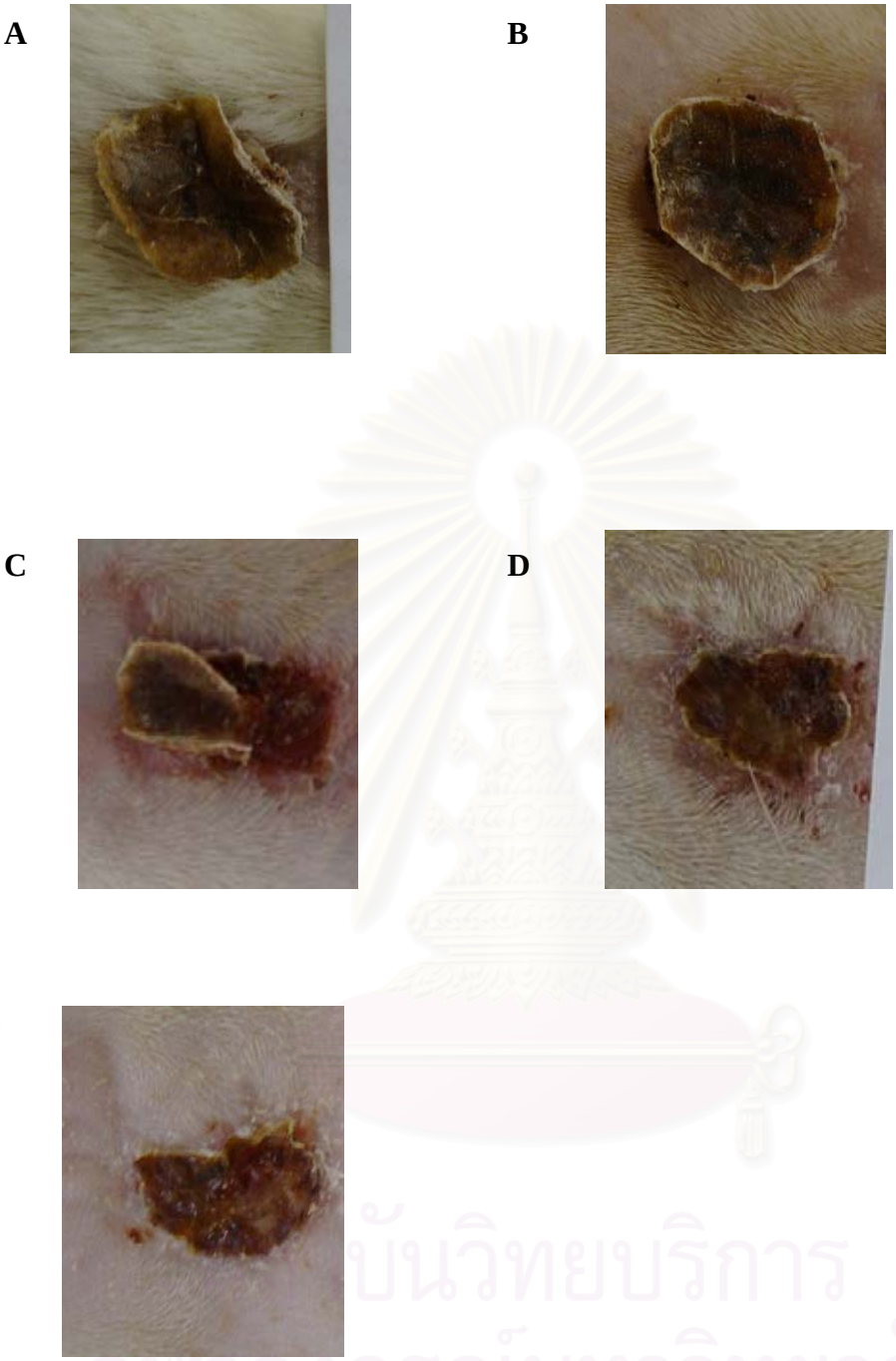


Figure 4.12 Burn wound on day 3 post burning in diabetic rats.

A) Untreated wound B) DM Burn+NSS

C) DM Burn+1%MC D) DM Burn+5%MC

E) DM Burn+10%MC

4.3 Skin blood flow (Laser Doppler Flowmeter measurements)

4.3.1 Non-Diabetic rats

4.3.1.1 Skin blood flow on day 3

Skin blood flow measured at the wound, on day 3 after wounding at the center and surround area see Figure 3.6

Skin blood flow on day 3 post burning , The skin blood flow of each group (228.08 ± 16.04% , 236.65 ± 27.69% , 236.75 ± 26.21% , 212 ± 12.30% in NSS , 1%MC , 5%MC , 10%MC respectively) were significantly different from untreated group (125.70±12.05%). (Figure 4.13)

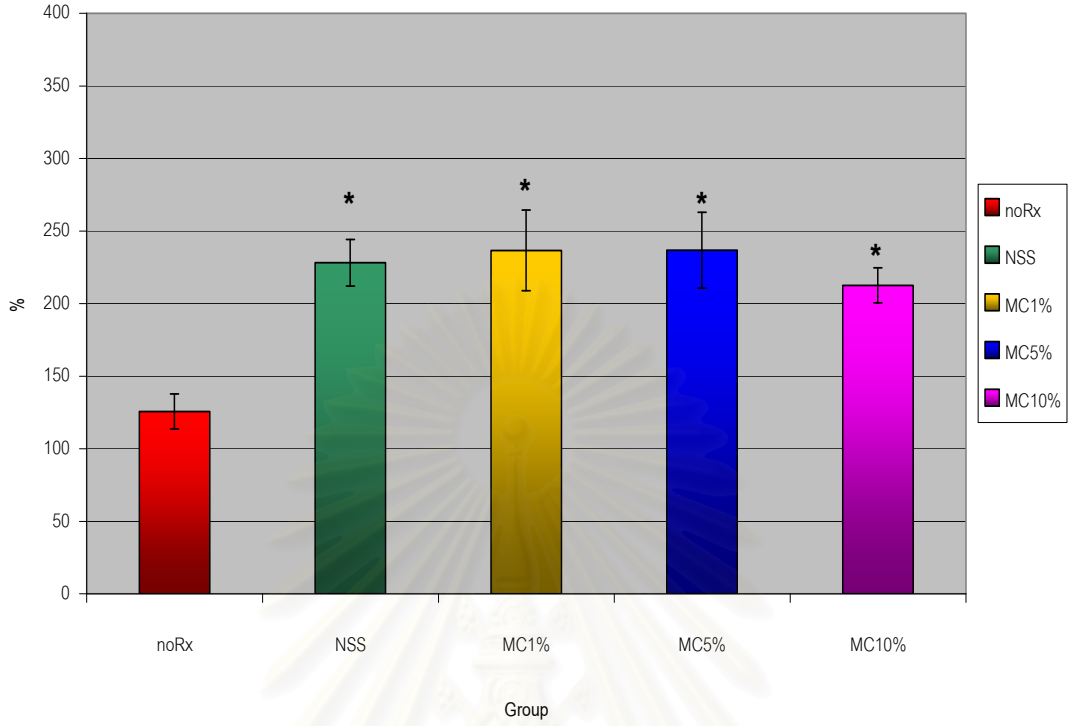


Figure 4.13 Skin blood flow on day 3 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

4.3.1.2 Skin blood flow on day 7 (Figure 4.14)

On day 7 post burning, skin blood flow of untreated group (124.62±10.49), NSS-treated (151.33 ± 25.03%) 10%MC (151.67 ± 25.03%) were significant different as compared to 1%MC (242.42 ± 10.94%) and 5%MC group (221.11 ± 14.01%)

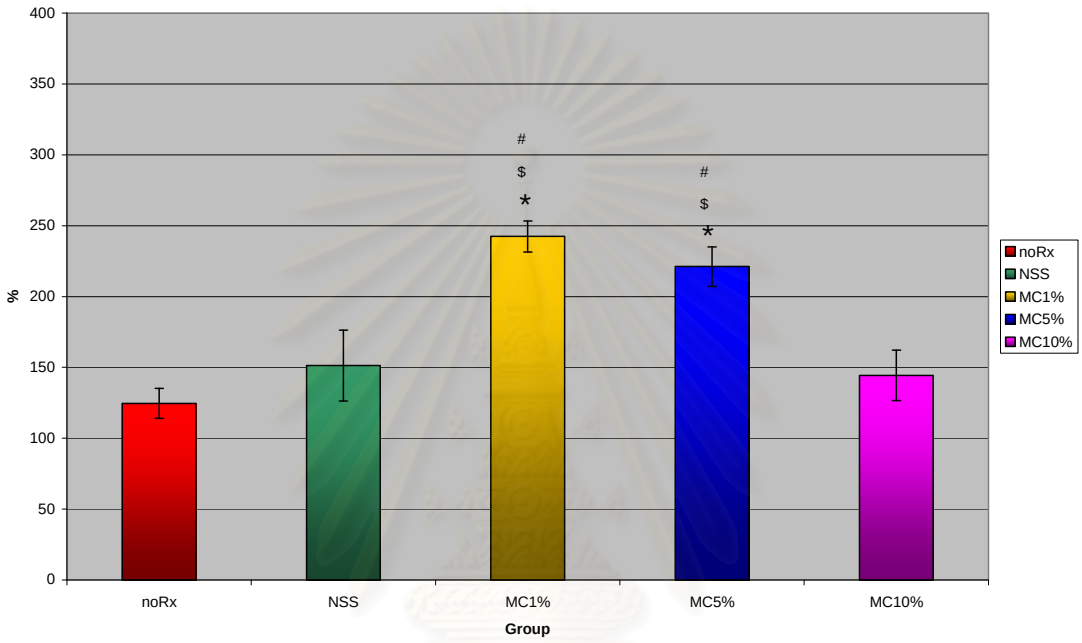


Figure 4.14 Skin blood flow on day 7 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

Significant difference as compared to 10%MC group ($p<0.05$)

3.1.2 Skin blood flow on Day 14 post burning

The skin blood flow of untreated group (76.98±8.47%) was not significant different as compared to each group (91.78 ± 11.11%, 103.17 ± 13.30%, 106.18 ± 6.24% , 86.98 ± 10.48% in NSS 1%MC , 5%MC , 10%MC treated group respectively) (Figure 4.15)

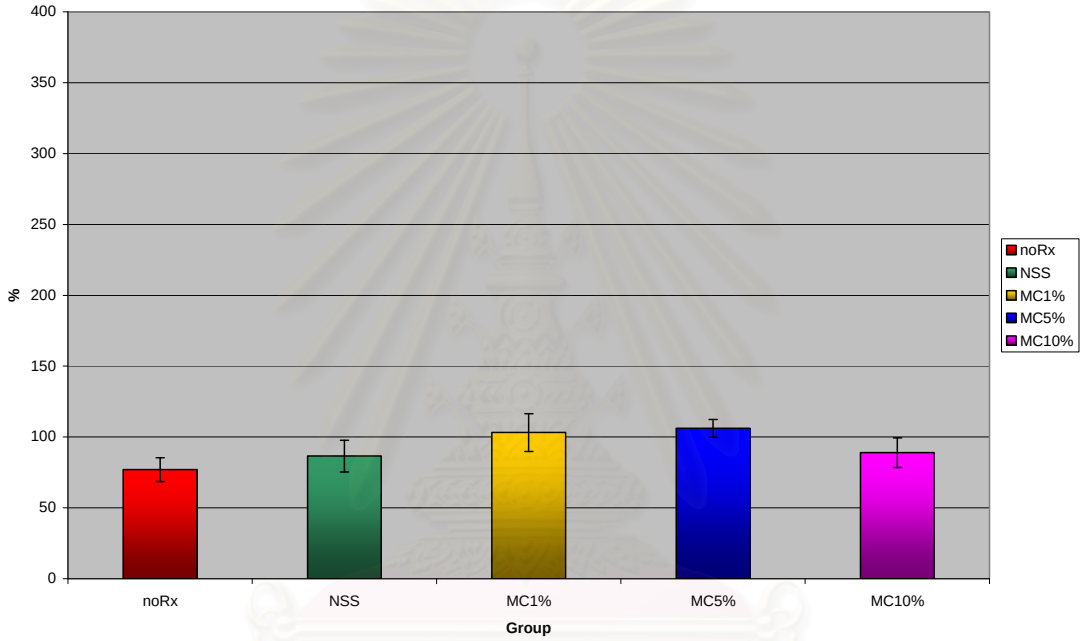


Figure 4.15 Skin blood flow on day 14 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

4.3.2 DM rat

4.3.2.1 Skin blood flow on day 3

On day 3 posts burning, skin blood flow of untreated group (58.51±8.87%) was not significant different from NSS-treated group. The skin blood flow of 1%MC (179.22± 21.76%) and 5%MC (151.86±27.13%) different as compared to untreated and NSS-treated group (69.57±21.76%). Furthermore, 1%MC was significant different as compare to 10%MC (212.61±12.03) (Figure 4.16).

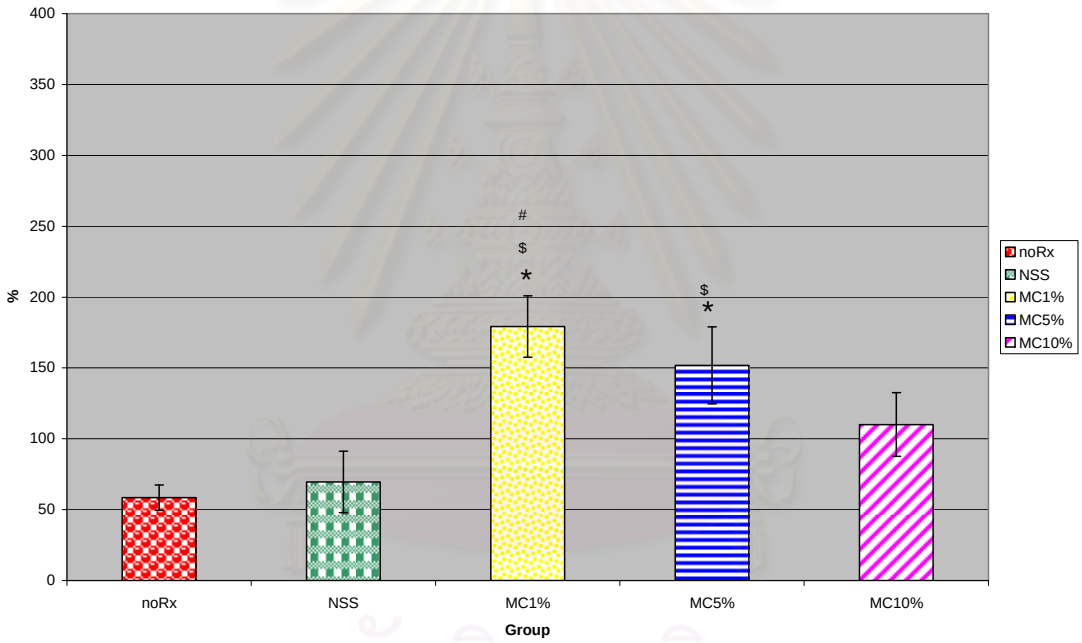


Figure 4.16 Skin blood flow on day 3 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

Significant difference as compared to 10%MC group ($p<0.05$)

4.3.2.2 Skin blood flow on Day 7

On day 7 post burning in diabetes rats .The skin blood flow of 1%MC (230.75 ±25.50%) was significant different compared to untreated (145.46±13.34%) and NSS-treated group(142.16±8.24%). The skin blood flow of untreated was not significant different from NSS treated. (Figure 4.17)

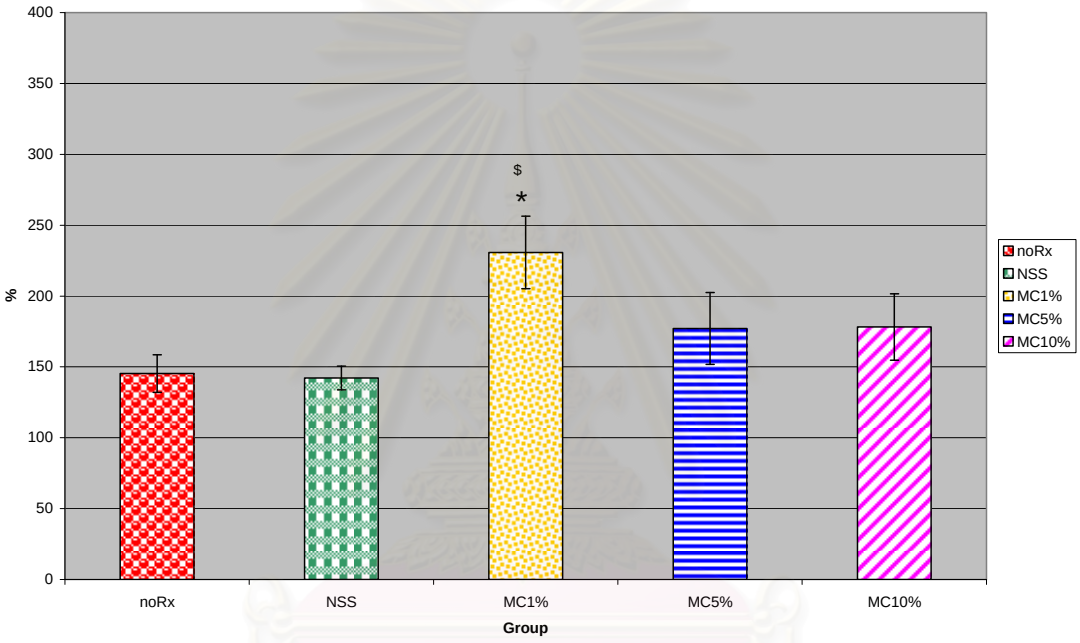


Figure 4.17 Skin blood flow on day 7 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

4.3.2.3 Skin blood flow on Day 14 post burning

On day 14 post burning in diabetes rats, the skin blood flow of untreated (132.91±16.45%) was not significant different from NSS treated (141.51 ±22.02%). 1%and 5%MC extract (296.55±39.71%, 277.95±39.75%) were significant different from untreated group. (Figure 4.18)

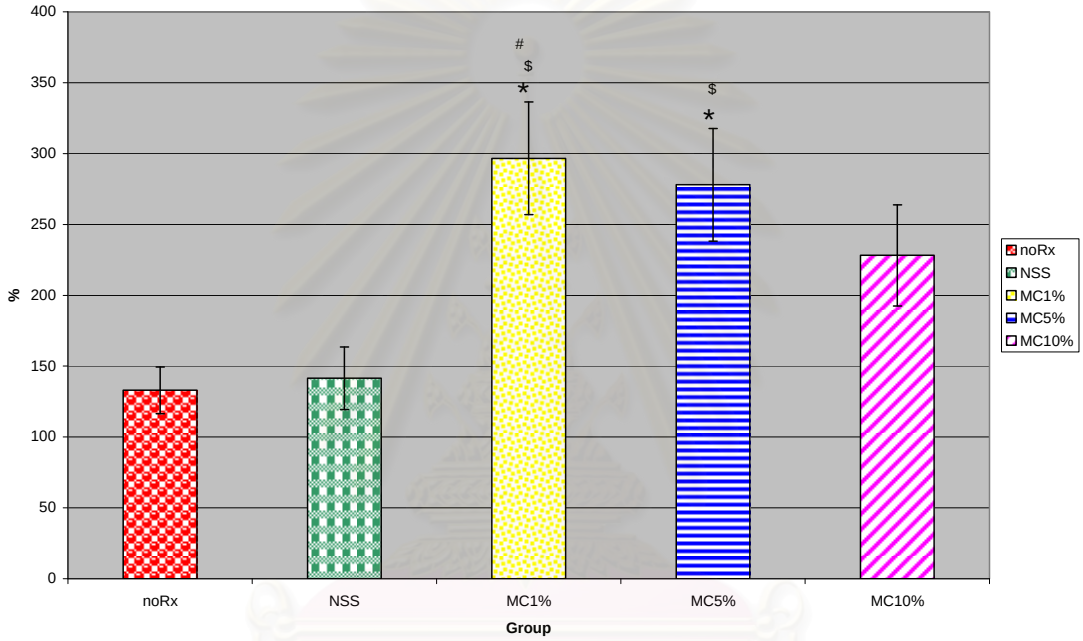


Figure 4.18 Skin blood flow on day 14 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

$ Significant difference as compared to NSS-treated group ($p<0.05$)

4.4 Lipid peroxidation assay

4.4.1 Non-Diabetic rat

4.4.1.1 Lipid peroxidation on days 3

The level of MDA in skin after burn injury on day 3, the group of 1%MC (4.87 ± 0.88 nmol/g tissue), 5%MC (5.94 ± 0.79 nmol/g tissue) and 10% (6.14 ± 2.29 nmol/g tissue) was significant different as compared to untreated group (9.21 ± 1.01 nmol/g tissue). However, 1% MC, 10%MC, 5%MC and untreated group were not significant different from NSS-treated (7.21 ± 0.77 nmol/g tissue). Our results showed that MC extract inhibit of burn injury induced MDA production in the wound sites. (Figure 4.19)

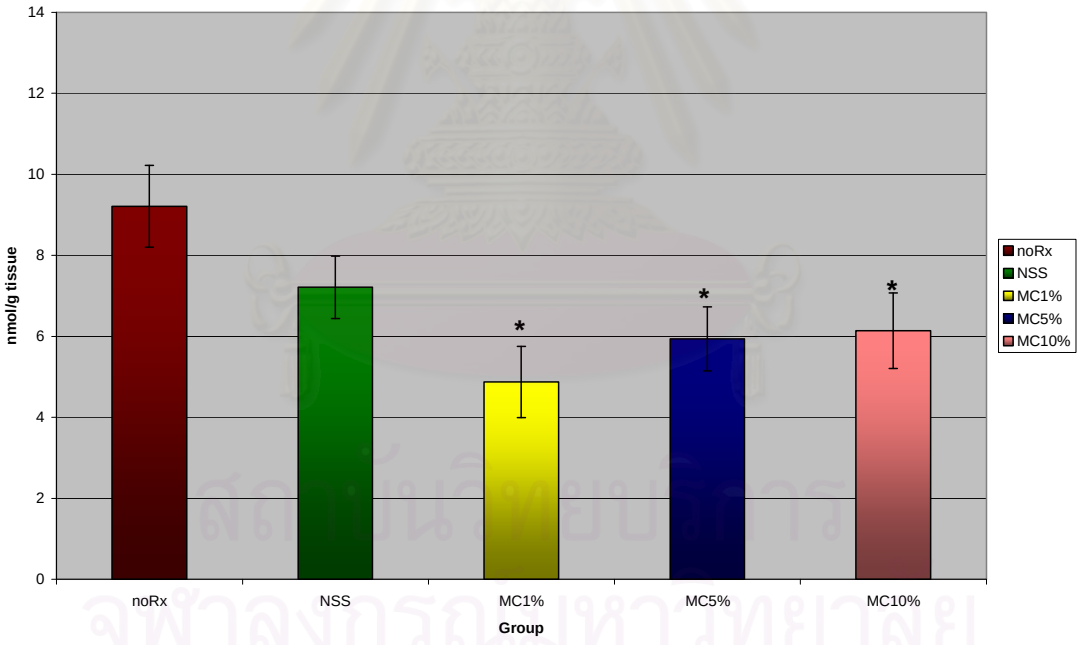


Figure 4.19 MDA level on day 3 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

4.4.1.2 Lipid peroxidation on days 7

The level of MDA in skin after burn injury on days 7. The group of 1%, 5%, 10%MC and NSS- treated group (6.13±0.76, 7.50±0.41, 8.62±0.84, 8.32±0.69 nmol/g tissue) were significant different as compared to untreated group (11.81±1.11 nmol/g tissue), similar to day 3.

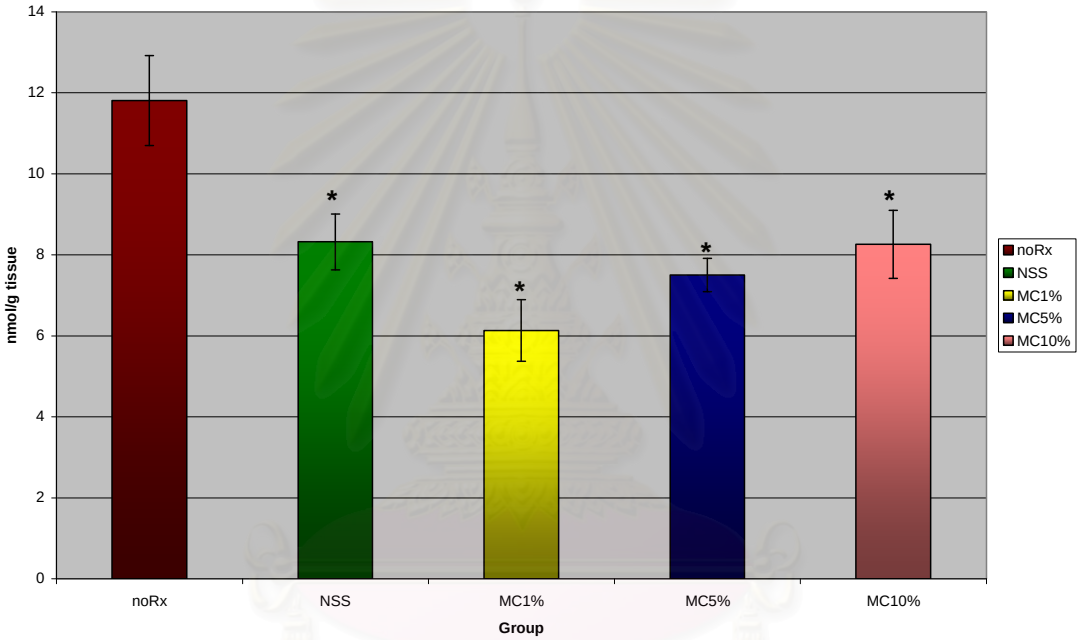


Figure 4.20 MDA level on day 7 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

4.4.1.3 Lipid peroxidation on days 14 in burn wound

The level of MDA in skin after burn injury on day 14, MDA level of each group were not significant different (6.85 ± 0.28, 6.41 ± 0.67, 5.86 ± 0.54, 5.91 ± 0.29, 6.06 ± 0.73 nmol/g tissue in untreated, NSS-treated, 1%MC, 5%MC, 10%MC) (Figure 4.21).

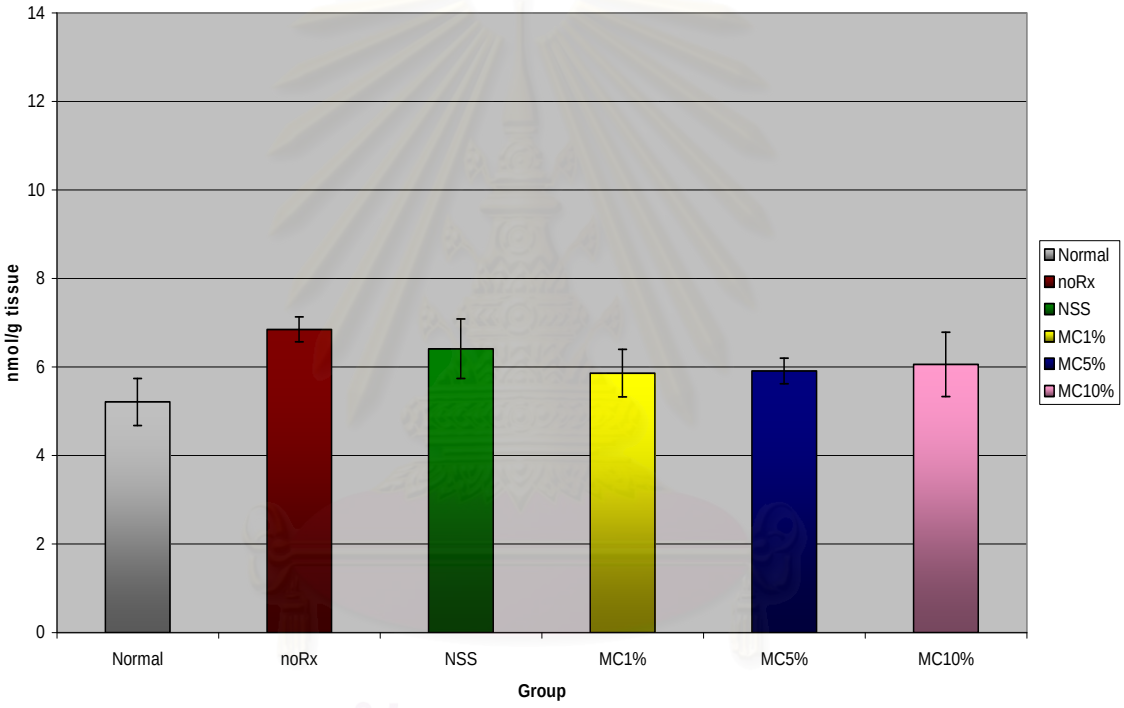


Figure 4.21 MDA level on day 14 post burning in non-diabetic rats.
noRx = untreated
NSS = Normal saline solution
MC = aqueous extract of *Malvastrum coromedelianum* Linn.
Values are means ± SEM; n=6

4.4.2 DM rat

4.4.2.1 Lipid peroxidation on days 3

The level of MDA in skin after burn injury on day 3, MDA level of each group were not significant different as compared to untreated group(8.16 ± 2.11, 6.71 ± 0.86, 4.45 ± 0.41, 4.71 ± 0.85, 5.74 ± 0.49 nmol/g tissue in untreated, NSS-treated, 1%MC , 5% MC, 10% MC treated group respectively).

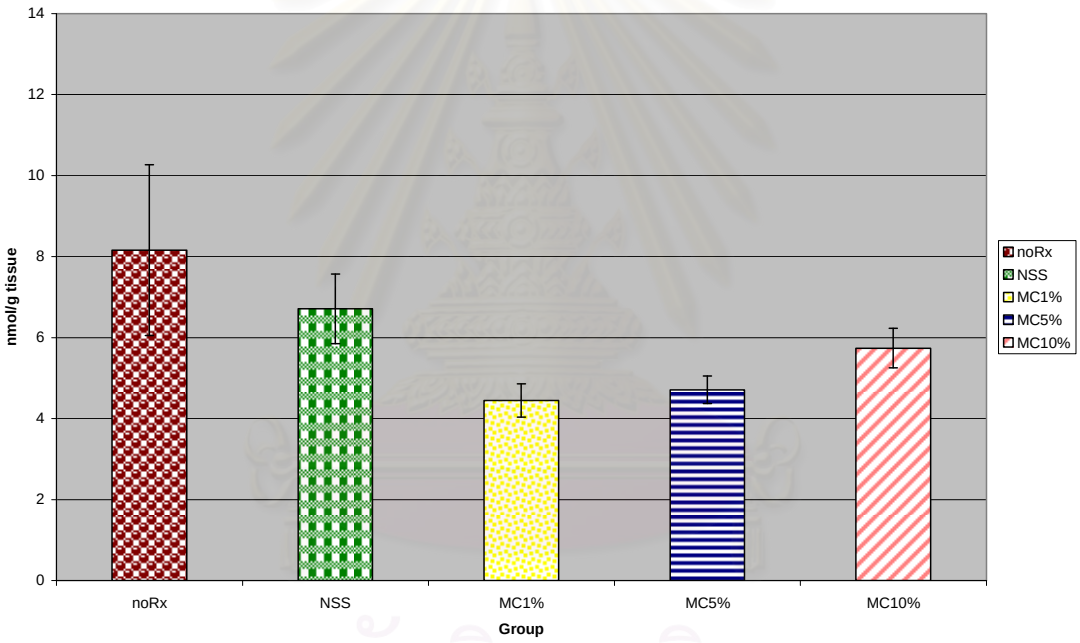


Figure 4.22 MDA level on day 3 post burning in diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

4.4.2.2 Lipid peroxidation on days 7

The level of MDA in skin after burn injury on day 7, MDA level of each group were not significant different as compared to untreated group(8.00 ± 0.36, 8.17 ± 1.07, 6.62 ± 0.39, 7.29 ± 0.94, 8.88 ± 1.11 nmol/g tissue in untreated, NSS-treated, 1%MC, 5%MC, 10% MC). (Figure 4.23)

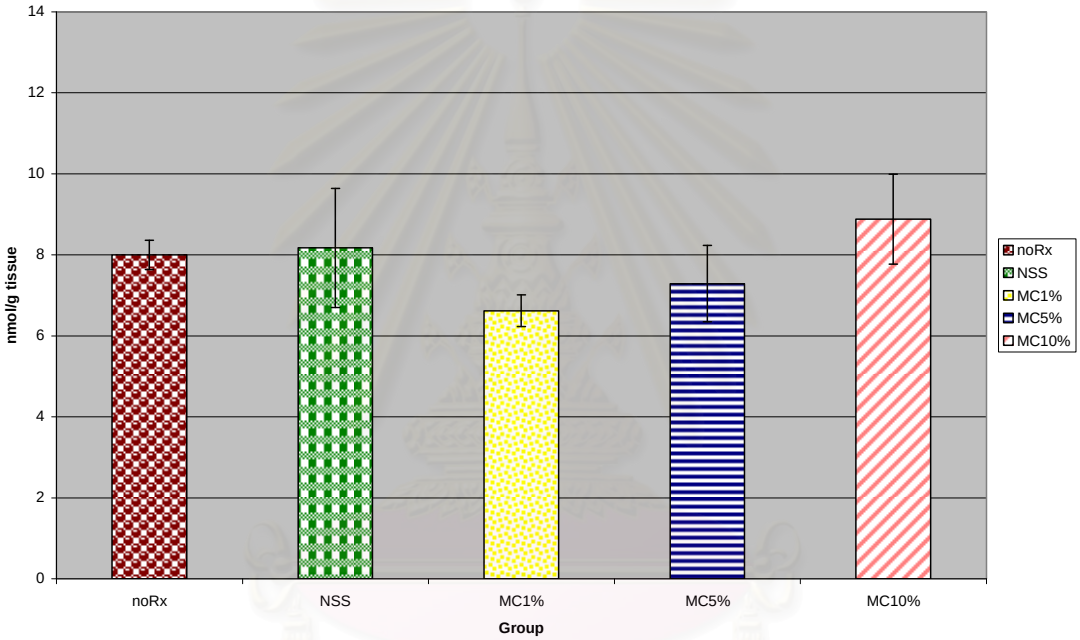


Figure 4.23 MDA level on day 7 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

4.4.2.3 Lipid peroxidation on days 14

The level of MDA in skin after burn injury on day 14, 1%MC (4.09 ± 0.75 nmol/g tissue), 5%MC(4.36 ± 0.44 nmol/g tissue),and 10%MC (4.91 ± 1.13 nmol/g tissue)group were significant different as compared to untreated group(9.71 ± 1.82 nmol/g tissue). However, untreated group were not significantly different from NSS-treated group (8.01 ± 1.83 nmol/g tissue). (Figure 4.24)

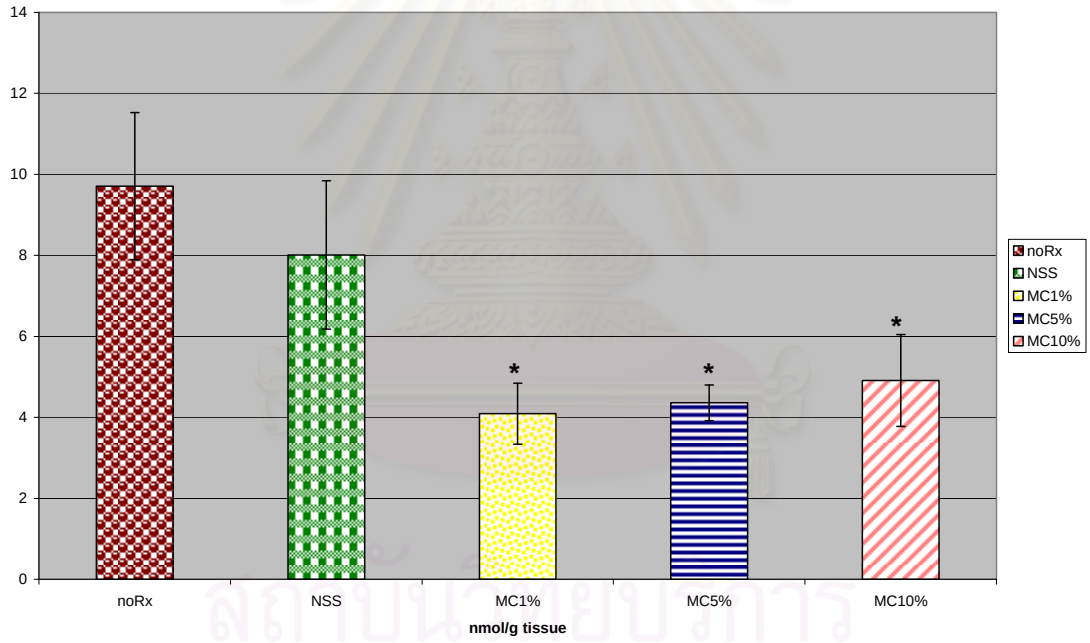


Figure 4.24 MDA level on day 3 post burning in non-diabetic rats.

noRx = untreated

NSS = Normal saline solution

MC = aqueous extract of *Malvastrum coromedelianum* Linn.

Values are means ± SEM; n=6

* Significant difference as compared to untreated group ($p<0.05$)

4.4 Histopathological observation

Histopathological evaluation of wound healing in this study was examined at day 3, 7 and 14 posts burning.

4.5.1 Histropathological observation of non diabetic rats

On day 3 post burning, the untreated and NSS-treated groups, the burn wound showed increased of inflammatory cell infiltrate distributed across the adipose tissue and necrosis of blood vessels. The wound in MC extract groups showed on vasculitis and fewer neutrophil (Figure 4.25).

On day 7 post burning, the untreated and NSS-treated groups have damage in epidermis and dermis and MC extract groups showed a prominent angiogenesis, fibroblast and keratinocytes migrate in to the wound bed from surrounding tissue. There was no inflammation (Figure 4.26).

On day 14 post burning, the MC extract groups showed fully developed epithelialization and keratinization. Skin appendages can be observed near to normal skin (Figure 4.27).

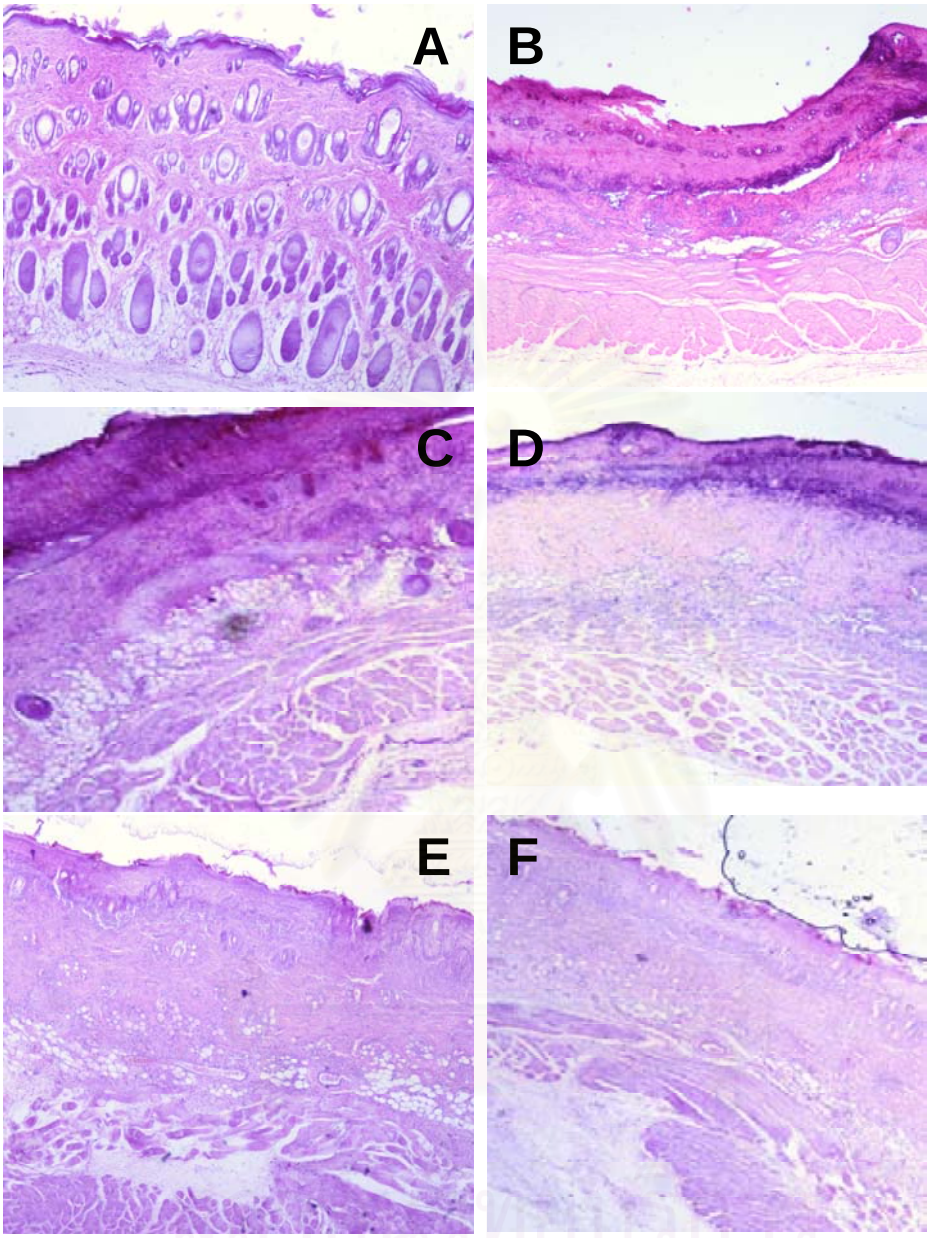


Figure 4.25 Hematoxylin-eosin stains. Histrological change of skin section on day 3 post burning in non-diabetic rat A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

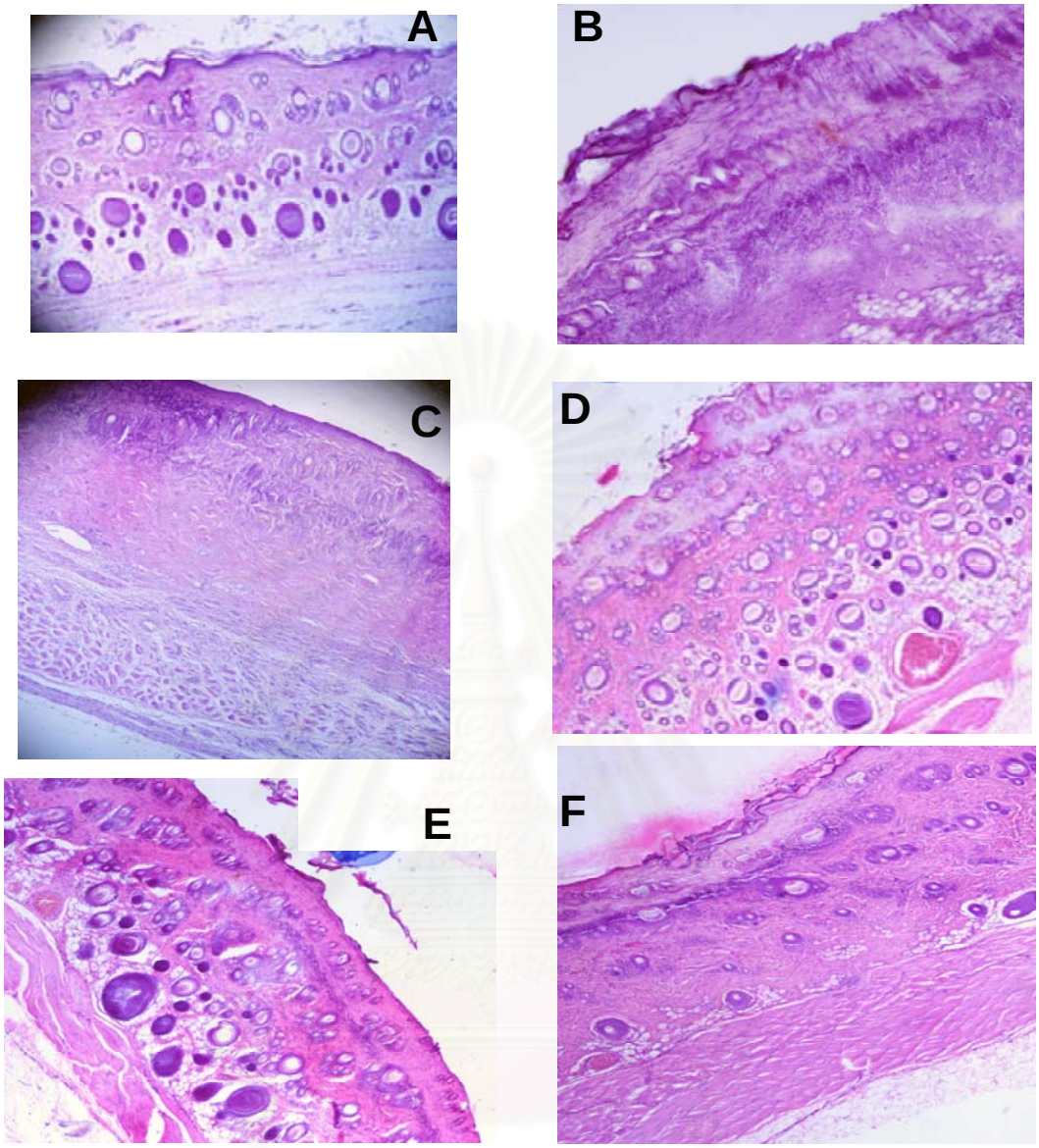


Figure 4.26 Hematoxylin-eosin stains. Histrological change of skin section at day 7 post burning in non-diabetic rat A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

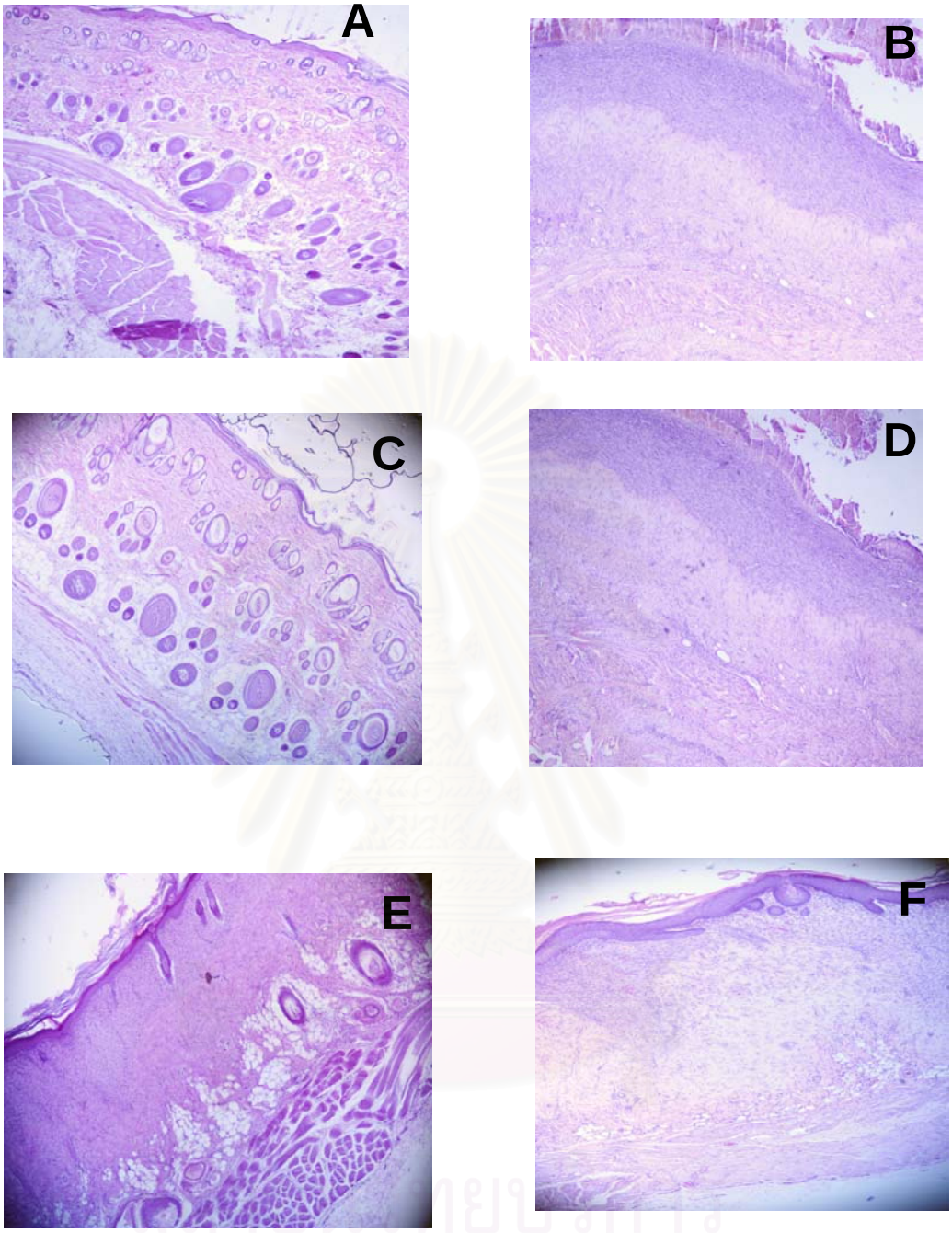


Figure 4.27 Hematoxylin-eosin stains. Histrological change of skin section at day 14 post burning in non-diabetic A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

4.4.2 Histopathological observation of DM rats

Histopathological evaluation of wound healing in this study was examined on day 3, 7 and 14 post burning in diabetic rats.

On day 3 post burning, the untreated and NSS-treated groups, the burn wound showed increased of inflammatory cells infiltrate distributed across the adipose tissue and also in the underlying skeletal muscle tissue, necrosis of blood vessels, infiltration of neutrophils in wound. The wound in MC extract group showed no vasculitis and fewer neutrophils (Figure 4.28).

On day 7 post burning, the untreated and NSS-treated group, there have damage of epidermis, dermis, fewer neutrophils and MC extract groups showed a prominent angiogenesis, fibroblast and keratinocytes migrate in to the wound bed from surrounding tissue. There no inflammation (Figure 4.29).

On day 14 psost burning, the MC extract group showed fully developed epithelialization and keratinization (figure 4.30).

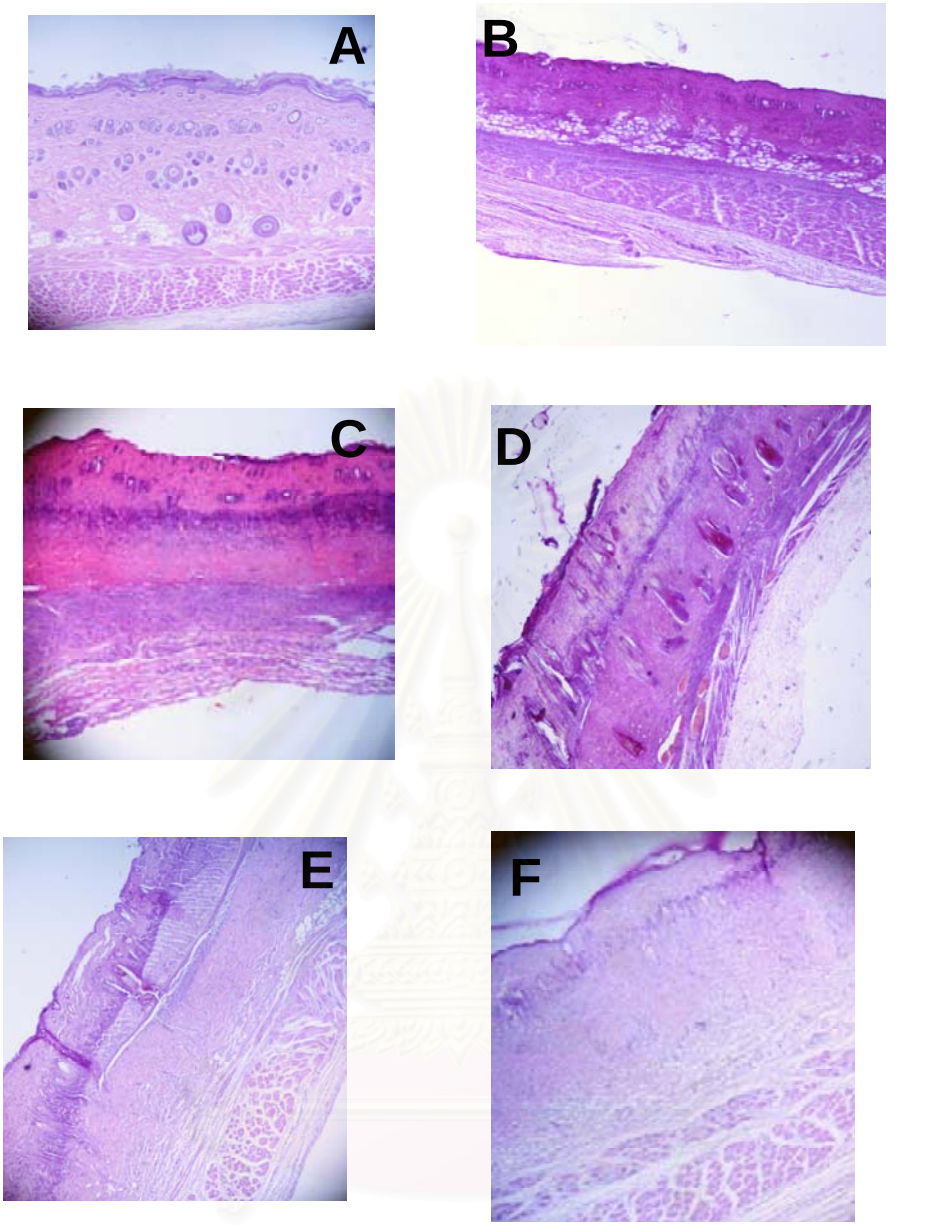


Figure 4.28 Hematoxylin-eosin stains. Histrological change of skin section at day 3 post burning in DM rat A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

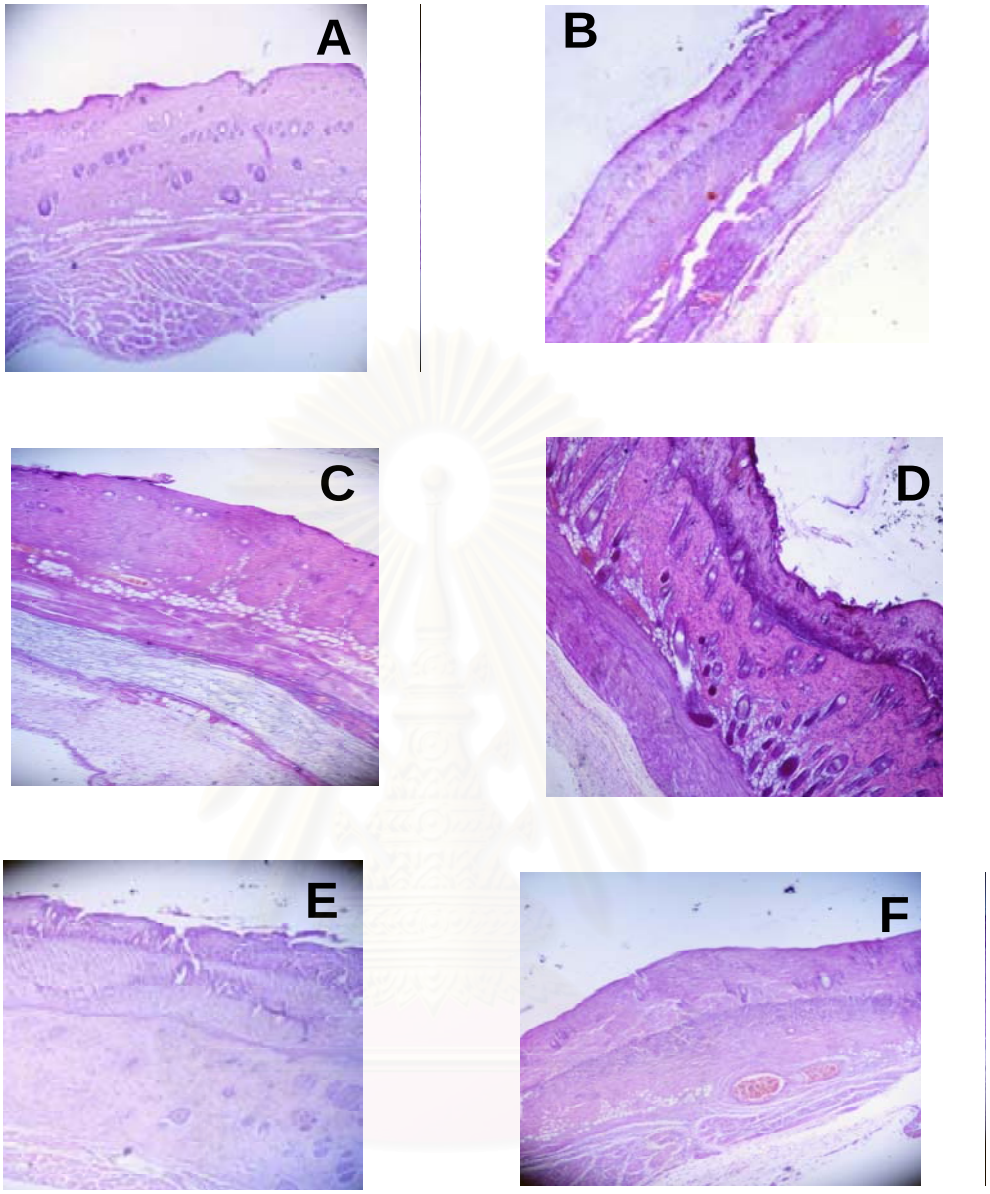


Figure 4.29 Hematoxylin-eosin stains. Histrological change of skin section at day 7 post burning in DM rat A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

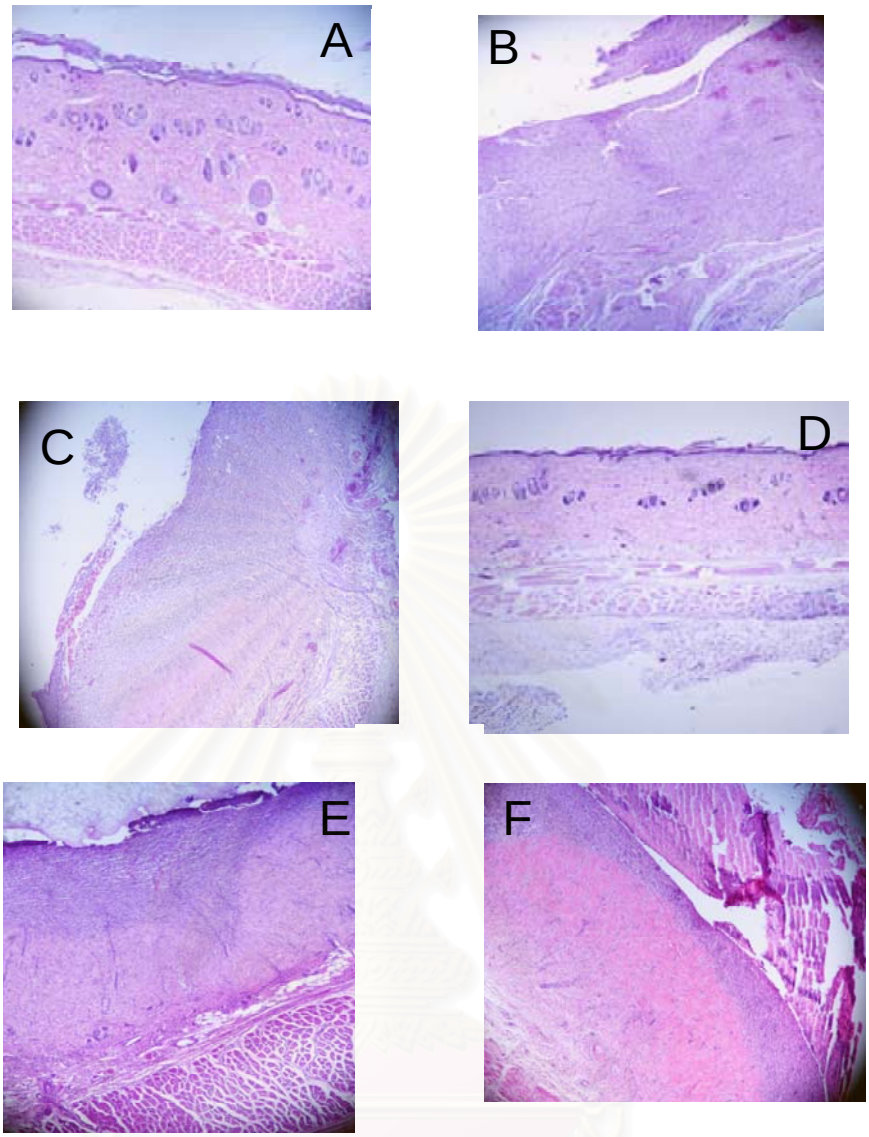


Figure 4.30 Hematoxylin-eosin stains. Histrological change of skin section at day 14 post burning in DM rat A) normal skin, B) untreated wound, C) wound treated with NSS-treated wound, D) wound treated with 1%MC extract, E) wound treated with 5%MC extract, F) wound treated with 10%MC extract

CHAPTER V

DISCUSSION

The present studies aimed to investigate the effect of topically applied extract of (MC) on burn wound of both normal and STZ-induced diabetic rats. Degree of wound healing, measurement of cutaneous blood flow and MDA level as well as histopathological studies were performed at day 3, 7 and 14 post wounding.

In burn wound blood, microcirculation is compromised to the worst extent at around 12-24 hours post wounding (Zawacki, 1974). Damage of blood vessels in the area of the wound caused restriction or cessation of blood flow which in turn causing ischemia and subsequently a reperfusion and thus a generation of oxidative stress (Ward and Till, 1900; Youn et al, 1992; LaLonde et al, 2003). Intervention that could restore blood flow or scavenging the free radicals should, in principle, attenuate damage due to oxidative stress (Mallikajuna, 2002).

In normal rats, on day 3 post burning, it was found that despite an increase in cutaneous blood flow in all groups, degree of wound healing in 1 and 5 % MC treated groups was significantly higher than those of other groups. Contraction of wound edge was noted. The wound surface was rather dry and showed very mild degree of swelling whereas prominent swelling of all layers (epidermis, dermis and subcutaneous) and exudates were apparent in untreated and NSS treated groups. Sign of inflammation in untreated and NSS treated groups was also evident in microscopic evaluation in which vasculitis and distribution of neutrophil in all layers were clearly observed. In contrast, no vasculitis and fewer neutrophil were observed in MC-treated groups. Decreased signs of inflammation in MC-treated rats could be ascribed to anti-inflammatory effect of MC previously reported by Moulun et al. (1999) as well as anti-oxidant properties which counteracted burn-induced oxidative stress resulting in a significant reduction of MDA level in all MC-treated group.

On day 7 post burning, increased blood flow seen in all groups of treatment in day 3 was maintained only in 1 and 5 % MC treated groups whereas the oxidative stress was significantly reduced in NSS-treated and in all MC extract-treated groups when comparing to untreated group. Discrepancy in responses observed suggests dissociation between active components responsible for anti-oxidative and vascular activity of MC extract. In correlation with responses of blood flow, degree of wound healing was significantly higher in 1 and 5 % MC treated groups. Increase in cutaneous blood flow by MC extract should provide adequate perfusion and subsequently oxygen and nutrient essential for wound healing process. Kloppenberg et al (2000) have shown that all burns that healed within 1 or 2 weeks showed an initial increased perfusion. Together with anti-oxidant properties and anti-inflammatory previously mentioned, 1 and 5 % MC extract treated group demonstrated positive effects on wound healing seen as a higher degree of wound contraction, hair formation on the edge of wound and collagen formation was noted. Thus the MC-treated wound seems to be in the late proliferative phase whereas untreated and NSS-treated groups remained in a late inflammatory phase as indicated by the presence of neutrophil in all layers of the skin, though to a lesser extent than those observed in day 3. Conclusively inflammatory phase was shortened by the application of MC extract.

On day 14 post burning, though the degree of wound healing in 1, 5 and 10% MC extract-treated groups were rather similar and significantly higher than those of untreated and NSS treated rats , complete closure of wound in conjunction with complete re-epithelialization was exclusively noted in 1% MC extract-treated group, thus, remodeling phase is expected (Hinz, 2005). As re-epithelialization in 5 and 10% MC extract-treated rats were incomplete, however, with fully grown dermis layer and no granulation tissue was noted, it is likely that both groups were in the late phase of proliferation (Midwood et al, 2004). In contrast, while re-epithelialization was rather scant, massive of granulation tissue was observed in dermis layer of untreated and NSS treated rats indicating early phase of proliferation (Midwood et al, 2004). Despite different phases of wound healing were estimated; as oxidative stress was subsided

(not different from unwound skin) blood flow was then normalized in all experimental groups (Fagrell, 1995; Rosenberg et al, 2006).

Interestingly, rather similar profile of responses to MC extract was observed in STZ-induced diabetic rats. Topical application of 1 and 5% MC extract was found to significantly increased blood flow. Estimation of degree of wound healing demonstrated that 1% of MC extract was found to act from a very beginning of wound healing process and continued throughout the course of experiment whereas the effects of 5 and 10% of MC extract were noted in day 3 and 7 post burning. However, progression of wound healing processes as reflected by signs of inflammation, characteristics of tissue observed, formation of collagen, completeness of epithelialization indicated a delay of responses in diabetic rats. At day 3, 7 and14, higher degree and longer duration of inflammation than their respective counterpart non-diabetic rats, was observed in diabetic rats. For example at day 14 while no neutrophil was observed in any (even untreated group) of non-diabetic rats, it was found consistently in all group of diabetic rats. Moreover at day 14 where complete closure in conjunctions with complete re-epithelialization was demonstrated in non-diabetic rats receiving 1%MC extract, incomplete closure together with incomplete re-epithelialization was elicited by the same concentration of MC in diabetic rats. Therefore the wound healing effect of MC extract was confirmed in diabetic rats. A delay in responses observed is likely to be due to malfunction of wound healing process previously described in diabetes. Furguson et al (1996) observed an increase in inflammatory cells, absence of cellular growth and lack of migration of the epidermis over the wound together with narrowing of blood vessel at the edge of the wound as well as impaired leucocyte function resulting in delayed wound healing in diabetic patients.

Furthermore, the fact that hyperglycemia-induced oxidative stress ultimately leads to tissue damage (King and Brownlee, 1966; Nishikawa, 2000; Brownless, 2001) might explain our finding that, unlike the response seen in non-diabetic rats, in diabetic rats oxidative stress induced by burning was not different from that of unwound animals. Anti-oxidant properties of MC extract were not evident in diabetic rats at day 3 and 7 but

on day 14 post burning. Protection of newly formed tissues from hyperglycemia- and/or burn injury-induced oxidative stress of MC extract could be attributable to the finding on day 14.

CHAPTER VI

Conclusion

In conclusion topical application of aqueous extract of MC clearly facilitated wound healing in both non-diabetic and diabetic rats. Shortening of inflammatory process by its anti-inflammatory effect, decreasing of oxidative injury by anti-oxidant property and increase of blood flow are plausible mechanisms underlying the wound healing observed.

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APPENDIX

1. Degree of wound healing

1.1 Non-DM rats

1.1.1 On day 3 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	14.00 ± 1.91	2.87 ± 0.06	6.49 ± 0.40	1.73 ± 0.06	1.96 ± 0.05
NSS	20.50 ± 4.05	2.65 ± 0.13	6.33 ± 0.17	1.70 ± 0.05	1.96 ± 0.07
1%MC	33.83 ± 3.34	2.21 ± 0.01	5.84 ± 0.14	1.50 ± 0.09	1.80 ± 0.03
5%MC	27.00 ± 2.16	2.43 ± 0.06	6.03 ± 0.09	1.70 ± 0.04	1.83 ± 0.05
10%MC	20.33 ± 3.12	2.66 ± 0.01	6.31 ± 0.11	1.62 ± 0.07	2.03 ± 0.08

1.1.2 On day 7 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	39.83 ± 3.34	2.01 ± 0.11	5.66 ± 0.18	1.45 ± 0.06	1.71 ± 0.07
NSS	43.16 ± 3.80	1.90 ± 0.12	5.49 ± 0.17	1.39 ± 0.03	1.64 ± 0.11
1%MC	63.83 ± 4.14	1.22 ± 0.13	4.47 ± 0.24	1.13 ± 0.09	1.41 ± 0.13
5%MC	56.50 ± 5.31	1.45 ± 0.17	4.98 ± 0.18	1.31 ± 0.08	1.31 ± 0.13
10%MC	48.00 ± 4.98	1.74 ± 0.16	5.63 ± 0.45	1.38 ± 0.08	1.37 ± 0.08

1.1.3 On day 14 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	73.50 ± 5.43	0.90 ± 0.18	4.09 ± 0.46	0.95 ± 0.16	1.11 ± 0.20
NSS	81.83 ± 3.36	0.62 ± 0.11	3.11 ± 0.30	0.80 ± 0.11	0.87 ± 0.11
1%MC	95.16 ± 2.50	0.17 ± 0.08	1.19 ± 0.55	0.29 ± 0.13	0.28 ± 0.13
5%MC	96.83 ± 1.44	0.11 ± 0.05	1.15 ± 0.44	0.26 ± 0.10	0.24 ± 0.10
10%MC	95.16 ± 3.52	0.16 ± 0.11	1.09 ± 0.58	0.20 ± 0.10	0.34 ± 0.19

1.2 DM rats

1.2.1 On day 3 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	12.33 ± 2.18	2.92 ± 0.07	6.83 ± 0.11	1.63 ± 0.06	2.03 ± 0.07
NSS	15.83 ± 6.19	2.81 ± 0.20	6.49 ± 0.23	1.67 ± 0.08	1.92 ± 0.06
1%MC	28.16 ± 3.84	2.40 ± 0.12	6.37 ± 0.33	1.65 ± 0.09	1.73 ± 0.06
5%MC	22.16 ± 2.72	2.59 ± 0.09	6.39 ± 0.18	1.60 ± 0.04	1.92 ± 0.06
10%MC	23.83 ± 3.88	2.54 ± 0.12	6.32 ± 0.19	1.63 ± 0.02	1.92 ± 0.07

1.2.2 On day 7 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	19.66 ± 1.47	2.69 ± 0.05	6.44 ± 0.09	1.73 ± 0.40	1.80 ± 0.04
NSS	20.83 ± 3.28	2.64 ± 0.11	6.49 ± 0.16	1.75 ± 0.07	1.78 ± 0.05
1%MC	37.66 ± 2.61	2.08 ± 0.08	5.91 ± 0.16	1.54 ± 0.03	1.57 ± 0.08
5%MC	30.50 ± 4.19	2.31 ± 0.13	6.25 ± 0.30	1.66 ± 0.04	1.63 ± 0.05
10%MC	31.00 ± 3.54	2.30 ± 0.11	5.94 ± 0.15	1.62 ± 0.06	1.68 ± 0.03

1.2.3 On day 14 post burning

Group	Degree of wound healing (%)	Area (cm^2)	Perimeter (cm)	Wide (cm)	Length (cm)
noRx	55.50 ± 3.07	1.48 ± 0.12	4.84 ± 0.21	1.33 ± 0.06	1.35 ± 0.05
NSS	57.83 ± 3.82	1.41 ± 0.12	4.73 ± 0.22	1.20 ± 0.05	1.35 ± 0.11
1%MC	74.16 ± 2.49	0.88 ± 0.08	3.92 ± 0.24	0.89 ± 0.07	1.27 ± 0.08
5%MC	74.00 ± 3.02	0.88 ± 0.10	3.82 ± 0.22	0.96 ± 0.07	1.04 ± 0.06
10%MC	75.16 ± 3.29	0.84 ± 0.10	3.96 ± 0.12	0.81 ± 0.05	1.07 ± 0.16

2. Cutaneous blood flow

2.1 Non-DM rats

2.1.1 On day 3 post burning

Group	Skin blood flow (AU)
Normal	78.02 ± 3.62
noRx	98.08 ± 9.40
NSS	177.92 ± 12.54
1%MC	184.64 ± 21.60
5%MC	184.68 ± 20.45
10%MC	165.88 ± 17.68

2.1.2 On day 7 post burning

Group	Skin blood flow (AU)
Normal	80.33 ± 8.60
noRx	100.11 ± 8.43
NSS	121.54 ± 14.23
1%MC	194.74 ± 20.75
5%MC	177.93 ± 20.11
10%MC	121.84 ± 11.48

2.1.3 On day 14 post burning

Group	Skin blood flow (AU)
Normal	73.02 ± 6.29
noRx	56.21 ± 6.18
NSS	68.21 ± 7.97
1%MC	75.34 ± 9.71
5%MC	77.54 ± 4.58
10%MC	64.98 ± 7.65

2.2 DM rats

2.2.1 On day 3 post burning

Group	Skin blood flow (AU)
Normal	47.37 ± 9.45
noRx	27.72 ± 4.02
NSS	32.96 ± 6.40
1%MC	86.06 ± 10.53
5%MC	71.94 ± 12.85
10%MC	52.15 ± 10.64

2.2.2 On day 7 post burning

Group	Skin blood flow (AU)
Normal	38.47 ± 5.52
noRx	55.96 ± 5.11
NSS	54.69 ± 3.16
1%MC	88.77 ± 9.81
5%MC	68.11 ± 9.37
10%MC	69.84 ± 8.35

2.2.3 On day 14 post burning

Group	Skin blood flow (AU)
Normal	30.85 ± 1.33
noRx	41.02 ± 5.07
NSS	43.65 ± 6.79
1%MC	91.16 ± 12.12
5%MC	85.74 ± 12.26
10%MC	70.39 ± 11.01

3. Lipid peroxidation

3.1 Non-DM rats

3.1.1 On day 3 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	4.13 ± 0.65
noRx	9.21 ± 1.01
NSS	7.21 ± 0.77
1%MC	4.87 ± 0.88
5%MC	5.94 ± 0.79
10%MC	6.14 ± 0.93

3.1.2 On day 7 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	4.53 ± 0.78
noRx	11.81 ± 1.11
NSS	8.32 ± 0.69
1%MC	6.13 ± 0.76
5%MC	7.50 ± 0.41
10%MC	8.62 ± 0.84

3.1.3 On day 14 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	5.21 ± 0.53
noRx	6.85 ± 0.28
NSS	6.41 ± 0.67
1%MC	5.86 ± 0.54
5%MC	5.91 ± 0.29
10%MC	6.06 ± 0.73

3.2 DM rats

3.2.1 On day 3 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	4.64 ± 1.02
noRx	8.16 ± 2.11
NSS	6.71 ± 0.89
1%MC	4.45 ± 0.41
5%MC	4.71 ± 0.34
10%MC	5.74 ± 0.49

3.2.2 On day 7 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	3.59 ± 0.22
noRx	8.00 ± 0.36
NSS	8.17 ± 1.47
1%MC	6.62 ± 0.39
5%MC	7.29 ± 0.94
10%MC	8.88 ± 1.11

3.2.3 On day 14 post burning

Group	Lipid peroxidation (nmol/g tissue)
Normal	3.11 ± 0.26
noRx	9.71 ± 1.82
NSS	8.01 ± 0.75
1%MC	4.09 ± 0.75
5%MC	4.36 ± 0.44
10%MC	4.91 ± 1.32

BIOGRAPHY

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