ผลของสารสกัดดายขัด (*MALVASTRUM COROMANDELIANUM* LINN.) ต่อการสมานแผลที่เกิดจากการกรีดในหนูขาวที่เป็นและไม่เป็นเบาหวาน

นางสาวรัชนีกร พานิชกรณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF *MALVASTRUM COROMANDELIANUM* LINN. EXTRACT ON INCISION WOUND HEALING IN DIABETIC AND NON-DIABETIC RATS

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Thesis Title	EFFECTS OF *MALVASTRUM COROMANDELIANUM* LINN. EXTRACT ON INCISION WOUND HEALING IN DIABETIC AND NON-DIABETIC RATS
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รัชนีกร พานิชกรณ์ : ผลของสารสกัดดายขัดต่อการสมานแผลที่เกิดจากการกรีดในหนูขาวที่เป็นและไม่เป็นเบาหวาน (EFFECTS OF *MALVASTRUN COROMANDELIANUM* LINN. EXTRACT ON INCISION WOUND HEALING IN DIABETIC AND NON-DIABETIC RATS) **อ.ที่ปรึกษา: รศ. ดร.บุญยงค์ ตันติสิระ, อ.ที่ปรึกษาร่วม รศ.ดร.มยุรี ตันติสิระ, รศ.พญ.จุไรพร สมบุญวงศ์, 70 หน้า**

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดดายขัดต่อการสมานแผลที่เกิดจากการกรีดในหนูขาวที่เป็นและไม่เป็นเบาหวาน โดยทำการศึกษาในหนูขาวเพศผู้ 120 ตัว น้ำหนักระหว่าง 250-300 กรัม แบ่งหนูออกเป็น 2 กลุ่ม กลุ่มละ 60 ตัว ได้แก่ หนูปกติ (ไม่เป็นเบาหวาน) และหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวาน โดยใช้ streptozotocin ในขนาด 50 มิลลิกรัมต่อกิโลกรัมฉีดเข้าทางหลอดเลือดดำที่หาง สัตว์ทดลองทั้งสองกลุ่มจะถูกแบ่งออกเป็น 5 กลุ่มย่อยได้แก่ กลุ่มที่ไม่ได้รับสารทดสอบใด ๆ กลุ่มที่ทาสารละลายน้ำเกลือ กลุ่มที่ทาสารสกัดดายขัด 1%, 5% และ 10% วันละครั้ง ที่บริเวณแผล ศึกษาผลของสารทดสอบในวันที่ 3 และ 7 หลังการทำให้เกิดแผล โดยวัดค่าแรงดึงสูงสุดที่ทำให้แผลเยื่บแยกออกจากกัน วัดค่าปริมาณคอลลาเจน วัดค่าความหนาของชั้นหนังกำพร้า และศึกษาการเปลี่ยนแปลงทางจุลพยาธิวิทยา นอกจากนั้น ยังทำการศึกษาผลของสารสกัดดายขัดต่อฤทธิ์ในการต้านเชื้อแบคทีเรียในจานเพาะเลี้ยง โดยทดสอบฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดดายขัดความเข้มข้น 1% 5% และ 10% โดยวิธี Filter paper disk-agar diffusion เชื้อที่ทำการทดสอบ ได้แก่ *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* และ *Escherichia coli*

จากการทดลองพบว่า ค่าแรงดึงสูงสุดที่ทำให้แผลเยื่บแยกออกจากกันและค่าความหนาของชั้นหนังกำพร้าของกลุ่มหนูขาวที่เป็นเบาหวาน (7 วัน) และหนูขาวปกติ (3 วัน) หลังการทาด้วยสารสกัดดายขัด 1% เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มที่ไม่ได้รับสารทดสอบใด ๆ และกลุ่มที่ทาสารละลายน้ำเกลือ ในขณะที่ไม่พบความแตกต่างของปริมาณคอลลาเจนอย่างมีนัยสำคัญทางสถิติ จากการสังเกตลักษณะและการเปลี่ยนแปลงทางพยาธิวิทยา พบว่ากลุ่มที่ทาสารสกัดดายขัดมีแนวโน้มที่จะหายดีกว่า นอกจากนั้นยังพบว่าสารสกัดดายขัดที่ความเข้มข้น 1% 5% และ 10% ไม่สามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียทั้ง 4 ชนิดได้แสดงว่าฤทธิ์ในการสมานแผลดังกล่าวข้างต้นไม่ได้เกิดจากฤทธิ์ในฆ่าเชื้อแบคทีเรีย

จากผลการทดลองข้างต้นอาจกล่าวได้ว่า สารสกัดดายขัดสามารถช่วยสมานแผลที่เกิดจากการกรีดได้ ทั้งในหนูที่เป็นและไม่เป็นเบาหวาน โดยที่แผลในหนูกลุ่มเบาหวานจะหายช้ากว่า จากการที่สารสกัดดายขัดที่ความเข้มข้น 1% มีผลในการเพิ่มค่าความหนาของชั้นหนังกำพร้าและค่าแรงดึงสูงสุดที่ทำให้แผลเยื่บแยกออกจากกัน โดยไม่มีผลต่อปริมาณคอลลาเจน ดังนั้น การเพิ่มความแข็งแรงของแผลดังกล่าวมาข้างต้น จึงอาจเป็นผลจากการที่สารสกัดดายขัดมีฤทธิ์เพิ่มสารอื่นที่ไม่ใช่คอลลาเจน แต่สามารถเพิ่มความแข็งแรงแก่ผิวหนังได้ ควรจะมีการศึกษาต่อไปถึงกลไกการออกฤทธิ์อื่น ๆ รวมทั้งสารสำคัญในสารสกัดดายขัดที่ออกฤทธิ์สมานแผล

สาขาวิชา สรีรวิทยา
ปีการศึกษา 2549

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: *MALVASTRUM COROMANDELIANUM* / WOUND HEALING / INCISION WOUND / DIABETIC

RATCHANEEKORN PANITCHAKORN: EFFECTS OF *MALVASTRUM COROMANDELIANUM* LINN. EXTRACT ON INCISION 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., THESIS CO-ADVISOR: ASSOC.PROF. JURAIPORN SOMBOONWONG, M.D.,M.Sc., 70 pages

The purpose of this study was to investigate the effect of an aqueous extract of *Malvastrum coromandelianum* on incision wound healing in diabetic and non-diabetic rats. Experiments were carried out in male Spraque-Dawley rats weighing 250-300 g. Rats were divided into 2 groups of 60 animals each for diabetic and non-diabetic rats models. Diabetes was induced by injection of Streptozotocin in the dose of 50 mg/kg B.W. intraveneously into tail vein. The animal in both groups were randomly subdivided into five sub groups as follow: untreated, NSS-treated, 1%, 5% and 10% of *Malvastrum coromandelianum* treated-group. Tested substances were applied topically once a day. On day 3 and 7 post wounding, the tensile strength, total collagen content, epidermal thickness and histopathological evaluation were assessed. In addition antibacterial activity of of *Malvastrum coromandelianum* (1%, 5% and 10%) was tested using filter paper disk-agar diffusion.

It was found that tensile strength and epidermal thickness of incision wound in diabetic group (day 7) and non-diabetic group (day 3) treated with 1% *Malvastrum coromandelianum* extract were significantly increased in comparison to those of untreated and NSS-treated groups while no significant increase of total collagen content was observed. In histopathological evaluation, the wound treated with *Malvastrum coromandelianum* extract tended to heal faster than those found in untreated and NSS-treated groups. Granulation as well as re-epithelialization were detected in herbal-treated groups earlier than those of untreated and NSS-treated groups. In addition none of 1%, 5% and 10% of *Malvastrum coromandelianum* extract was found to exhibit antibacterial activity against *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Escherichia coli*.

The present studies have demonstrated the wound healing effect of the aqueous extract of *Malvastrum coromandelianum* on incision wound in diabetic as well as non-diabetic rats though a slower rate of healing was noted in the diabetic group. An increase in epidermal thickness and positive effects on some other components than collagen of extracellular matrix exerted by 1% *Malvastrum coromandelianum* but not antibacterial activity seem to underlie an increase in tensile strength observed. Investigations to identify other possible mechanisms as well as active constituents of *Malvastrum coromandelianum* responsible for the wound healing effects should be further conducted.

Field of study Physiology
Academic year 2006

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LIST OF ABBREVIATIONS

%	Percent
β	Beta
μm	Micrometer
μg	Microgram
BM	Basement membrane
BW	Body weight
cm	Centimeter
cm^2	Square centimeter
°C	Degree Celsius
DM	Diabetes mellitus
e.g.	Exempli gratia (for example)
ECM	Extracellular matrix
EGF	Epidermal growth factor
Ep	Epidermis
F	Hair follicle
FGF	Fibroblast growth factor
G	Granulation tissue
gm	Gram
kg.	Kilogram
hrs	hours
H&E	Hematoxylin and eosin
L.	Liter
M	Mole
mg/dl	Milligram per deciliter
mg/kg	Milligram per kilogram
ml	Milliliter
min	Minute
mm	Millimeter

mm^2	Square millimeter
MC	*Malvastrum coromandelianum*
m^2	Square meter
nm	Nanometer
N/ cm^2	Newton per Square centimeter
NSS	Normal saline solution
PMNL	Polymorphonuclear leukocytes
S.E.M	Standard error mean
STZ	Streptozotocin
UV	Ultraviolet
v/v	Volume by volume
VEGF	Vascular endothelial growth factor
w/v	Weight by volume

CHAPTER I

INTRODUCTION

A wound is a break in the epithelium integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue. A wound may result from precise disruption of tissue by the surgeon's knife (incision) to widespread damage of tissue (e.g. major trauma, burns). A wound may also result from a contusion, heamatoma, laceration or an abrasion. (Enoch and Leaper, 2004)

Healing of wound occurs as a carefully regulated, systemic cascade of overlapping process that require the coordinated completion of a variety of cellular activities, including phagocytosis, chemotaxis, mitogenesis, and synthesis of component of the extracellular matrix. These activities occur in a cascade that correlate with the appearance of different cell types in the wound during various stages of the healing process. These processes involve four overlapping phases of haemostasis, inflammation, proliferation, remodeling and scar maturation (Enoch and Leaper, 2004; Williamson and Harding, 2004).

Diabetes mellitus is well known as being associated with altered healing. In diabetics, healing impairment is caused by several intrinsic factors (neuropathy, vascular problem, and other complicating systemic effects due to diabetes) and extrinsic factors (wound infection, callus formation, and excessive pressure to the site). Especially, the infection is an extremely important cause of morbidity and hospitalization, amputation and impaired wound. So, care should be focused on facilitating wound healing and preventing complications of existing wounds (Falanga, 2005).

Development of drugs or agents to treat wound has been continuously going on. Some agents are found to be useful in the treatment of wound such as: flucidic acid, the topical agent, has long been used in Thailand. It can inhibit gram-positive bacterial infection but its efficacy is associated with side effect of rash.

Mupirocin which is used to treat gram-negative bacterial infection is slowly absorbed and excreted thought kidney. The side effects of this agent are pain and irritation at wound site. It should be avoided in patient with renal failure. In addition, Povidone-iodine is another agent used to treat skin wound. This agent is effective against viral, bacterial and fungal infection without serious side effects.

Thailand has many kinds of herbal products which have long been used in traditional medicine. Various plants are recommended to be used in the household. Among these plants is *Malvastrum coromandelianum* Linn. (MC) Garcke, belonging to Malvaceae family, commonly found in Asia. It is known as Prickly malvastrum, Ya-tevada, Threelobe false mallow and Daikhad. MC has been used in traditional medicine in Asia such as Thailand, India. It has ability to cleanse wound, reduce blood glucose, microbial and carbuncles.

Though there are some studies on the effect of MC but there are no studies of its effect on wound. Thus, the purpose of this study was to investigate the effects of MC on incision wound healing in diabetic and non-diabetic rats.

Objective

1. To study the effects of MC extract on incision wound healing in diabetic and non-diabetic rats.

2. To study the antibacterial activity of MC extract.

CHAPTER II

LITERATURE REVIEWS

2.1 *Malvastrum coromandelianum* (Linn.) Garcke

MC is a perennial herb belonging to the family Malvaceae. It is locally known as Prickly malvastrum, Ya-tevada, Three-lobe false mallow, Daikhad. As shown in Figure 2.1, the stem is an erect, somewhat hairy, branched, half-woody perennial, 1 meter in height or less. The leaves are oblong to ovate-lanceolate, 2 to 5 cm long, with blunt tip and usually rounded base, and irregularly toothed margins. The flowers are axillary and terminal. The calyx is green, and about 7 mm long, with lanceolate pointed lobes. The petals are yellow, and about 8 mm long. The fruit consists of 8 to 12 reniform, compressed, hirsute carpel 2 to mm long, each carpel having 3 short, straight projections (Sebastainand and Bhandari, 1984; Jesadanont et al., 2005; Huang and Zhou, 2006).

Figure 2.1 *Malvastrum coromandelianum* (Linn.) Garck.

2.1.1 Traditional using (Reddy, Venkatesh and Suresh, 2001)

MC is a medicine plant. In India, MC are used for anti-inflammatory, analgesic, antidysenteric, jaundice and cleanse ulcers.

2.1.2 Pharmacological study

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

2.1.2.1 Antimicrobial activity

The water extract of MC had been tested for antimicrobial activity using Agar Disc Diffusion Susceptibility Test and Broth Macro Dilution test. In 2005, Pongpech *et al*. found that the 25% (w/v) of water extract had been shown to have inhibitory activity against both methicillin sensitive and methillin resistant *Staphylococcus aureus.*

2.1.2.2 Hypoglycemic activity

In 2005, Rattanajarasroj *et al.* has reported the hypoglycemic activity of MC in both normal and alloxan-induced diabetic rats. It was found that an oral administration of MC extract at the dose of 4 g/kg BW showed a significant hypoglycemic effect in alloxan-induced diabetic rats.

Water extract from whole plant of MC showed strong hypoglycemia activity when given orally. Feeding the crude extract to streptozotocin- induced male wistar rat, reduced blood glucose within one hour after administration to a level of blood glucose in normal non-diabetic rats. This strength hypoglycemic activity of this plants extract would fulfill the search for an oral hypoglycemia agent equivalent to insulin injection (Jesadanont *et al*, 2005).

2.1.2.3 Antinociceptive activity

In 2001, Reddy, Venkatesh and Suresh, has reported the aerial parts of MC showed antinociceptive activity in the 0.6% acetic acid-induced writhing test in mice.

2.1.2.4 Antipyretic, Analgesic and Anti- inflammatory

Antipyretic, Analgesic and Anti-inflammatory effects of MC were tested by fever caused by typhoid-paratyphoid vaccine in rabbit; hot-plate, writhing, ear edema, and abdominal capillary permeability of mice. The results showed that extract of MC could lower rabbit fever, decrease mice writhing, inhibit ear edema, and decrease the permeability of the blood capillary in abdominal cavity of mice (Moulum, Wen and Shiying, 1999).

2.2 The skin

2.2.1 Structure of the skin (Marieb, 1981; Mast, 1992; Falkel, 1994; Povell, 2006)

The skin consists of different tissues that are joined to perform specific functions (Figure 2.2). It is the largest organ of the body in surface area and weight. In adults, the skin covers an area of about 2 square meters and weight 4.5-5 kg, about 16% of total body weight. It ranges in thickness from 0.5 mm on eyelids to 1-2 mm thick. The skin is made up of three layers: epidermis, dermis and subcutaneous.

2.2.1.1 The epidermis

The epidermis is the outermost layer of the skin and protects the body from the environment. The thickness of the epidermis varies in different types of skin; it is only .05 mm thick on the eyelids, and is 1.5 mm thick on the palms and the soles of the feet. The epidermis contains the melanocytes (the cells in which melanoma develops), the Langerhans cells (involved in the immune system in the skin), Merkel cells and sensory nerves. The epidermis layer itself is made up of five sublayers that work together to

continually rebuild the surface of the skin: basal cell layer, squamous cell layer, stratrum granulosum, stratrum lucidum and stratrum corneum.

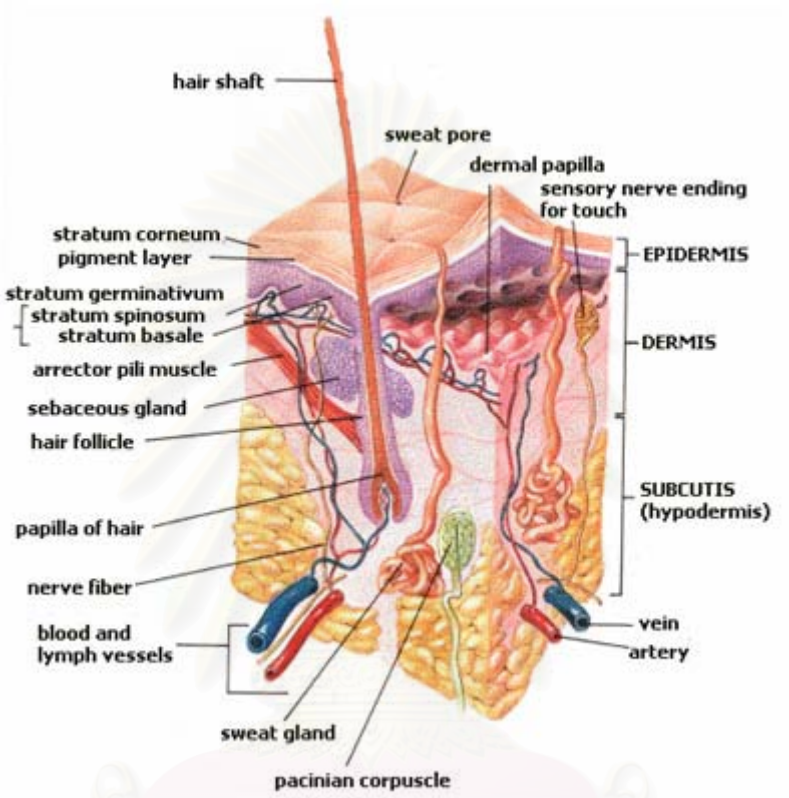

Figure 2.2 The skin

2.2.1.1.1 The Basal Cell Layer

The basal layer is the innermost layer of the epidermis, and contains small round cells called basal cells. The basal cells continually divide, and new cells constantly push older ones up toward the surface of the skin, where they are eventually shed. The basal cell layer is also known as the stratum germinativum due to the fact that it is constantly germinating (producing) new cells

. 2.2.1.1.2 The Squamous Cell Layer

The squamous cell layer is located above the basal layer, and is also known as the stratum spinosum or “spiny layer” due to the fact that the cells are held together with spiny projections. Within this layer are the basal cells that have been pushed upward, however these maturing cells are now called squamous cells, or keratinocytes. Keratinocytes produce keratin, a tough, protective protein that makes up the majority of the structure of the skin, hair, and nails. The squamous cell layer is the thickest layer of the epidermis, and is involved in the transfer of certain substances in and out of the body. The squamous cell layer also contains cells called Langerhans cells. These cells attach themselves to antigens that invade damaged skin and alert the immune system to their presence.

2.2.1.1.3 The Stratum Granulosum and the Stratum Lucidum

The keratinocytes from the squamous layer are then pushed up through two thin epidermal layers called the stratum granulosum and the stratum lucidum. As these cells move further towards the surface of the skin, they get bigger and flatter and adhere together, and then eventually become dehydrated and die. This process results in the cells fusing together into layers of tough, durable material, which continue to migrate up to the surface of the skin.

2.2.1.1.4 The Stratum Corneum

The stratum corneum is the outermost layer of the epidermis, and is made up of 10 to 30 thin layers of continually shedding, dead keratinocytes. The stratum corneum is also known as the “horny layer,” because its cells are toughened like an animal’s horn. As the outermost cells age and wear down, they are replaced by new layers of strong, long-wearing cells. The stratum corneum is sloughed off continually as new cells take its place, but this shedding process slows down with age. Complete cell turnover occurs every 28 to 30 days in young adults, while the same process takes 45 to 50 days in elderly adults.

2.2.1.2 The Dermis

The dermis is located beneath the epidermis and is the thickest of the three layers of the skin (1.5 to 4 mm thick), making up approximately 90 % of the thickness of the skin. The main functions of the dermis are to regulate temperature and to supply the epidermis with nutrient-saturated blood. Much of the body's water supply is stored within the dermis. This layer contains most of the skins' specialized cells and structures, including: blood vessels, lymph nodes, hair follicles, sweat glands (apocrine glands, eccrine glands), sebaceous glands, nerve endings, collagen and elastin. The dermis layer is made up of two sublayers: the papillary layer and the reticular layer.

2.2.1.2.1 The Papillary Layer

The upper, papillary layer contains a thin arrangement of collagen fibers. The papillary layer supplies nutrients to select layers of the epidermis and regulates temperature. Both of these functions are accomplished with a thin, extensive vascular system that operates similarly to other vascular systems in the body. Constriction and expansion control the amount of blood that flows through the skin and dictate whether body heat is dispelled when the skin is hot or conserved when it is cold.

2.2.1.2.2 The Reticular Layer

The lower, reticular layer is thicker and made of thick collagen fibers that are arranged in parallel to the surface of the skin. The reticular layer is denser than the papillary dermis, and it strengthens the skin, providing structure and elasticity. It also supports other components of the skin, such as hair follicles, sweat glands, and sebaceous glands.

2.2.1.3 Subcutaneous Layer

The subcutaneous layer, or superficial fascia, attaches the skin to underlying tissues and organs. It consists primarily of loose connective tissue and adipose tissue. Subcutaneous adipose tissue serves as a heat insulator and a storage site for fat. It

conserves body heat and retards the penetration of external heat into body. Blood vessels and nerves within the subcutaneous layer give off branches that supply the dermis

2.2.2 Function of the skin

The skin helps to regulate body temperature, serves as a water-repellent and protective barrier between the external environment and internal tissue, contains sensory nerve ending, excretes a small amount of salts and several organic compounds and has some capacity to absorb substance, and helps to synthesis the active form of vitamin D. We next briefly examine each of these functions.

2.2.2.1 Regulation of body temperature.

In response to high environmental temperature or strenuous exercise, the production of perspiration by soporiferous (sweat) glands helps lower body temperature back to normal. Changes in the flow of blood to the skin also alter its insulating properties and help to adjust body temperature.

2.2.2.2 Protection.

The skin covers the body and provides a physical barrier that protects underlying tissues from physical abrasion, bacterial invasion, dehydration, and ultraviolet (UV) radiation.

2.2.2.3 Reception of stimuli

The skin contains numerous nerve endings and receptors that detect stimuli related to temperature, touch, pressure, and pain.

2.2.2.4 Excretion

Not only does perspiration assume a role in helping to regulate normal body temperature, it also assists in the excretion of small almost of water, salts, and several organic compounds.

2.2.2.5 Synthesis of Vitamin D

Exposure to UV radiation converts precursor molecules in the skin into vitamin D.

2.2.2.6 Immunity

Certain cells of the epidermis are important components of immunity, your ability to fight disease by producing antibodies.

2.3 The Wound

2.3.1 Definition of wound

A wound is a break in the epithelium integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue. A wound may result from precise disruption of tissue by the surgeon's knife (incision) to widespread damage of tissue (e.g. major trauma, burns). A wound may also result from a contusion, heamatoma, laceration or an abrasion (Enoch and Leaper, 2004).

2.3.2 Types of wound (Leaper and Gottrup, 1998)

2.3.2.1 Incisions or incised wounds

Incised wounds involve no loss and minimal damage to tissue. There are two types, surgical incision and non-surgical wound.

2.3.2.1.1 Surgical incision are usually clean, except when made to treat an infective condition such as an abscess or fecal peritonitis. They are usually placed anatomically to avoid major vessels and nerves.

2.3.2.1.2 Penetrating, non-surgical wounds are caused by injuries inflicted by a knife or other sharp instrument.

2.3.2.2 Lacerations

Irregular wounds are caused by a blunt impact to soft tissue which lies over hard tissue (e.g. laceration of the skin covering the skull) or tearing of skin and other tissues such as caused by childbirth. Lacerations may show bridging, as connective tissue or blood vessels are flattened against the underlying hard surface.

2.3.2.3 Abrasions

Injury resulting in this type of wound is associated with loss of the superficial layers of epithelium. Nerve ending are exposed and the wounds are painful. When extensive, the blood or plasma loss may be substantial and mimic a burn injury.

2.3.2.4 Contusions

These wounds are a more severe form of laceration and follow a much greater energy exchange. The tissue layers are separated and there is often tissue loss leaving an open wound.

2.3.2.5 Ulcer

An ulcer may be defined as a loss of an epithelial surface together with a variable degree of underlying connective tissue. The majority of acute ulcers follow trauma or pyogenic infections and there is usually some loss of connective tissue. The defect is made good by wound contraction and epithelialization and formation of scar tissue in the subepithelial layers. The chronic ulcer is a lesion which fails to heal and its aetiology may be very diverse. The ulcer needs management of the underlying cause.

2.3.3 Wound healing (Standelman, Digenis and Tobin, 1998; Singer and Clark, 1999; Strodtbeck, 2001)

Wound healing is a physiological process involving a series of sequential yet overlapping stages. There are anywhere from 3 to 5 stage a of wound healing that merge into a continuous process as follows:

2.3.3.1 Hemostasis phase (immediate)

Tissue injury is characterized by microvascular injury and extravasation of blood into the wound. Loss of structural integrity initiates the coagulation cascade and constriction of vessel wall; the resulting clot formation and platelet aggregation limits further blood loss. The platelets trapped in the clot are essential for hemostasis and a normal inflammatory response. The platelets degranulate and release their granules, which secrete several factors, including platelet-derived growth factor, insulin-like growth factor-1, epidermal growth factor, transforming growth factor, and platelet factor-IV. These proteins initiate the wound healing cascade by attracting and activating fibroblasts, endothelials cell and

macrophages. These events also activate four major amplification systems (complement cascade, clotting mechanism, kinin cascade, plasmin generation), which contribute to hemostasis and the subsequent stages of the healing process. The clot provides the provisional matrix for cellular migration. The platelets also contain dense bodies that store vasoactive amines such as; serotonin, bradykinin, and histamine that increase microvascular permeability, leading to exudation of fluid into the extravascular space.

2.3.3.2 Inflammation phase (day 1-5)

The second stage of wound healing is inflammation. The inflammatory response is triggered by a variety of mediators released from injured tissue cells and capillaries, activated platelets and their cytokines, and the by-products of hemostasis. Within minutes after the injury, neutrophils arrive to contain any microorganisms present in the wound. Although other white cells, including monocytes, lymphocytes, and plasma cells, migrate to the injury site, in the absence of infection, the existing monocytes differentiate into macrophages and become the major phagocytic cell at the injury site. Macrophage infiltration predominates for the remainder of the wound healing process. In addition to ingesting surviving microorganisms, dead neutrophils, the fibrin clot, and other cellular debris, macrophages synthesize nitric oxide and secrete cytokines to initiate wound repair. Another important mechanism in the inflammatory stage of wound healing is activation of vasoactive substances such as serotonin, bradykinin, prostaglandins, and histamine. These substances increase permeability of endothelium within the injury site and increase perfusion to the site. Increased permeability facilitates infiltration by immune and repair cells, whereas increased circulation increases oxygen delivery. At the end of the inflammation stage of wound healing, bleeding is controlled and the wound bed is clean. This creates the perfect environment for the next stage of cell proliferation and repair.

2.3.3.3 Proliferative phase or regenerative phase (day 3-14)

The specific mechanisms of stage 3 are aimed at covering the wound surface with new skin (re-epithelialization), restoring vascular integrity to the region (neovascularization),

and repairing the structure integrity of the tissue defect by filling it with new connective tissue (granulation). Wound (tensile) strength begins to develop during this stage. Key cells for these processes are the fibroblast and keratinocyte.

The process of restoring the vascular network is called neovascularization or angiogenesis. Neovascularization is stimulated by growth factor and tissue hypoxia. Hypoxia is thought to induce macrophages into secreting angiogenesis. Platelets and macrophage-derived growth factors, such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), are released by endothelial cell and stimulate angiogenesis along the wound edges. New blood vessels bud or sprout from intact vessels in the underlying dermis. The new capillary buds join to form capillary loops thereby establishing blood flow within the wound. New sprout or buds extend from the capillary loop further into the wound environment. The process of arborization is thus stimulated by hypoxia within the wound environment and results in the growth factor of new vessels throughout the wound.

Re-epithelialization of a wound occurs when keratinocytes completely cover the surface of skin defect. Keratinocyts, stimulated by locally released growth factor, proliferate and begin their migration across the wound bed within 12- 24 hrs after injury. Because the site of proliferation is usually proximal to the injury, the new keratinocytes must migrate to the repair site. Migration proceeds from the wound edges towards the center in a centripetal manner. This process is repeated until the migrating cells from opposing sides of wound touch each other. Once migration is complete, the keratinocytes stabilize themselves by forming firm attachments to each other and the new basement membrane.

The third and final mechanism of proliferation and repair is the development of granulation tissue. Granulation tissue, a transitional substance that replaced the fibrin/fibronectin matrix, begins to appear about 4 days after injury. Granulation tissue occurs as the fibril clot scaffold is replaced with new tissue rich in hyaluronan, fibronectin, and other ECM compounds. Because granulation tissue is very active metabolically and

supports the proliferation of a variety of cell and protein, it is also highly vascular. This accounts for its classic pinkish-red appearance.

2.3.3.4 Maturation phase or remodeling phase (day 7 to 1 year)

The final stage of wound healing is remodeling or maturation of the granulation tissue into mature connective tissue and/or scar. The wound also develops its final strength during this stage of wound healing. The key cells for remodeling are macrophage and fibroblast. ECM reshaping by cross-linking collagens, cell maturation, and program cell death or apoptosis are the mechanism used in wound remodeling. Collagen synthesis peaks around 5 days after injury but continues for weeks or months. During this time, collagen and ECM tissue continue to be deposited into the wound, whereas the developing connective tissue is reshaped by cell maturation and apoptosis. Although wound strength increases, it never achieves more than 80% of the preinjury strength.

2.3.4 Types of wound healing (Halloran and Slavin, 2002; Rubin and Farber, 2004)

It is traditional to make a distinction between the healing of the opposed edges of a clean incised wound (healing by primary intention) and the separated edges of a gouged wound (healing by secondary intention)(Figure 2.3).

2.3.4.1 Healing by first intention (wounds with opposed edges)

The least complicated example of wound repair is the healing of a clean, uninfected surgical incision approximated by surgical sutures. Such healing is referred to as primary union or healing by first intention. The incision causes death of a limited number of epithelial cells and connective tissue cells as well as disruption of epithelial basement membrane (BM) continuity. The narrow incision space immediately fills with clotted blood containing fibrin and blood cells; dehydration of the surface clot forms the well-known scab that covers the wound.

Within 24 hrs, neutrophils appear at the margins of the incision, moving toward the fibrin clot. The epidermis at its cut edges thickens as a result of mitotic activity of basal cells, and within 24 to 48 hrs, spurs of epithelial cells from the edges both migrate and grow along the cut margins of the dermis, depositing BM component as they move. They fuse in the midline beneath the surface scab, thus producing a continuous but thin epithelial layer.

By day 3, the neutrophils have been largely replaced by macrophages. Granulation tissue progressively invades the incision space. Collagen fibers are now present in the margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epidermal covering layer.

By day 5, the incision space is filled with granulation tissue. Neovascularization is maximal. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness, and differentiation of surface cells yields mature epidermal architecture with surface keratinization.

During the second week, there is continued accumulation of collagen and proliferation of fibroblasts. The leukocytic infiltrate, edema, and increased vascularity have largely disappeared. At this time, the long process of blanching begins, accomplished by the increased accumulation of collagen within the incision scar, accompanied by regression of vascular channels.

By the end of the first month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate, covered now by intact epidermis. The dermal appendages that have been destroyed in the line of the incision are permanently lost. Tensile strength of the wound increases thereafter, but it may take months for the wounded area to obtain its maximal strength. Although most skin lesion heal efficiently, the end product may not be functionally perfect. Epidermal appendages do not regenerate, and there remains a dense connective tissue scar in place of the mechanically efficient meshwork of collagen in the unwounded dermis.

2.3.4.2 Healing by secondary intention (wounds with separated edges)

When there is more extensive loss of cells and tissue, as occurs in infarction, inflammatory ulceration, abscess formation, and surface wounds that create large defects, the reparative process is more complicated. The common denominator in all these situations is a large tissue defect that must be filled. Regeneration of parenchymal cells cannot completely reconstitute the original architecture. Abundant granulation tissue grows in from the margin to complete the repair. This form of healing is referred to as secondary union or healing by secondary intention. Secondary healing differs from primary healing in several respects:

1. Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudates that must be removed. Consequently the inflammatory reaction is more intense.

2. Much large amounts of granulation tissue are formed. When a large defect occurs in deeper tissues, such as in a viscous, granulation tissue with its numerous scavenger white cells bears the full responsibility for its closure because drainage to the surface cannot occur.

3. Perhaps the feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds. Large defects in the skin of rabbit are reduced in proximately 6 weeks to 5-10% of their original size, largely by contraction. Contraction has been ascribed, at least in part, to the presence of myofibroblasts-altered fibroblasts that have the ultra structural characteristics of smooth muscle cells.

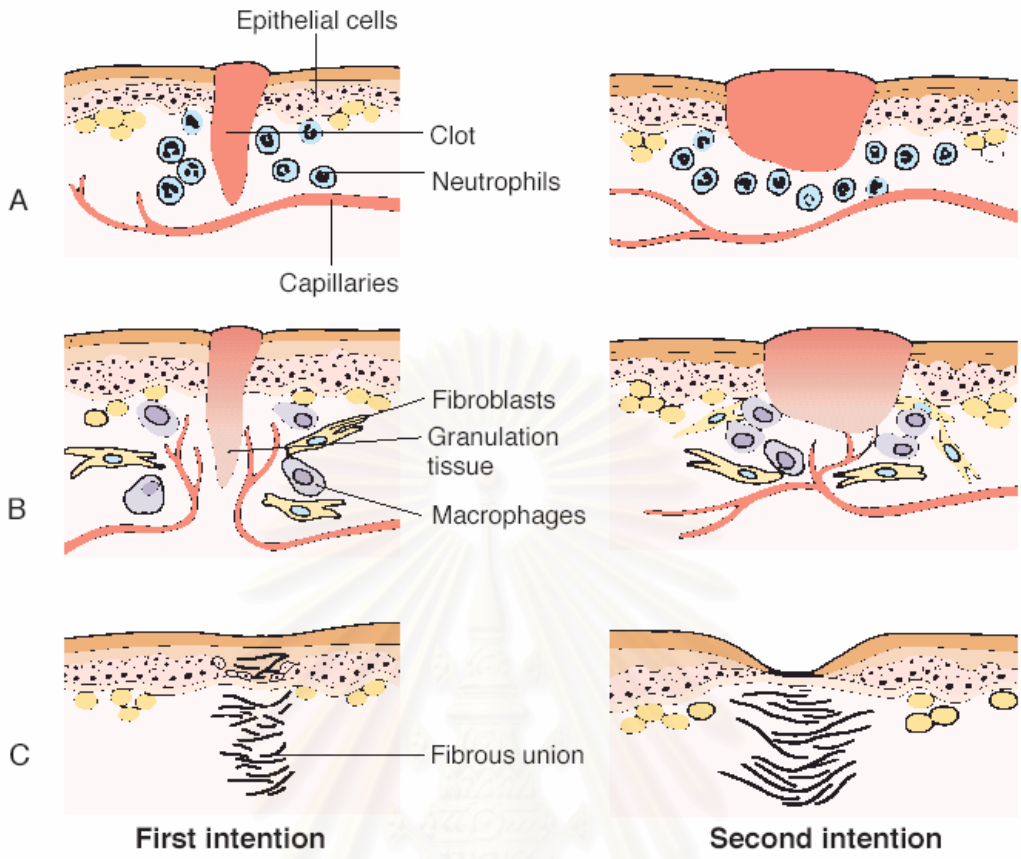

Figure 2.3 Steps in wound healing by first intention (left) and secondary intention (right). In the latter, the resultant scar is much smaller than the original wound, owing to wound contraction.

2.3.5 Factors that influence wound healing (Greenhalgh and Staley, 1994; Mulder *et al.*, 1998)

We have discussed the usual manifestations of repair, and we reviewed the orderly healing of wound in normal persons. But these processes are modified by a number of known influences and some unknown ones; frequently impair the quality and adequacy of both inflammation and repair. These influences include both local and systemic factors.

2.3.5.1 Local factors

2.3.5.1.1 Type, size, and location of the wound

A Clean, aseptic wound produced by the surgeon's scalpel heals faster than a wound produced by blunt trauma, which exhibits abundant necrosis and irregular edges. Small blunt wounds heal faster than larger ones. Injuries in richly vascularized areas heal faster than those poorly vascularized ones. In areas where the skin adheres to bony surfaces, as in injuries over the tibia, wound contraction and adequate apposition of the edges are difficult.

2.3.5.1.2 Vascular supply

Wounds with impaired blood supply heal slowly. For example, the healing of leg wounds in patients with varicose veins is prolonged. Ischemia due to pressure produces bedsores and then prevents their healing. Ischemia caused by arterial obstruction, often in the lower extremities of diabetics, also prevents healing.

2.3.5.1.3 Infection

Gross bacterial infection of a wound certainly delays or even reverses the healing response. Local destruction of tissue by bacterial growth and enzymatic action as well as prolongation of the inflammatory phase of healing is responsible. However, the abundance

of normal bacteria flora of the skin suggests that virtually all wounds are contaminated to some degree. Yet contamination does not always lead to infection or deficient healing.

2.3.5.1.4 Movement

Early motion, particularly before tensile strength has been established, subjects a wound to persistent trauma, thereby preventing or retarding healing.

2.3.5.1.5 Ionizing radiation

Prior irradiation leaves vascular lesions that interfere with blood supply and result in slow wound healing. Acutely, irradiation of a wound blocks cell proliferation, inhibits contraction, and retards the formation of granulation tissue.

2.3.5.1.6 Ultraviolet light

Exposure of wounds to UV light accelerates the rate of healing.

2.3.5.2 Systemic factors

2.3.5.2.1 Circulatory status

Cardiovascular status, by determining the blood supply to the injured area, is important for wound healing. Poor healing attributed to old age is often due largely to impaired circulation.

2.3.5.2.2 Malnutrition

Severe protein malnutrition affects body protein metabolism and thus may exert effects on collagen synthesis and connective tissue deposition. Moreover, the severely malnourished patient is immunosuppressed, thus affecting the inflammatory phase of

healing. Local control of bacterial contamination may not be as efficient, leading to an increased likelihood of wound infection. This suppression can also blunt the proliferative phase of healing as macrophage infiltration and the cytokines are reduced.

2.3.5.2.3 Vitamin and trace elements

Deficiencies in vitamins and trace elements also cause clinical healing problems.

2.3.5.2.4 Diabetics

Diabetes mellitus (DM) is well known as being associated with altered healing. The effects of DM on the body are multiple, as are the effects on healing such as alteration on the function of leukocytes in such a way that chemotaxis, phagocytosis, and intracellular bacterial killing are all diminished. These effects would be expected to lead to impaired healing due to a less efficient inflammatory response, and the altered function of neutrophils, macrophages, and lymphocytes can cause diminished fibroblast proliferation and collagen deposition.

2.3.5.2.5 Hormones

Corticosteroids impair wound healing, an effect attributed to inhibition of collagen synthesis. However, these hormones also have many other effects, including anti-inflammatory actions and a general depression of protein synthesis. Thyroid hormones, androgens, estrogens, and growth hormone also influence wound healing. Their effects, however, may be due more to their regulation of general metabolic status than to a specific modification of the healing process.

2.4 Wound strength

There are variation that depends on the site of the wound, the species, and the depth of the incision. Carefully sutured wounds have approximately 70% of the strength of

unwounded skin. The strength of the healed wound depends on the deposition of an adequate extracellular matrix. The extracellular matrix has five major components: collagen, basement membranes, elastic fiber, glycoprotein, and proteoglycans (Rubin and Farber, 2004).

2.4.1 Collagen

Collagen is one of the major components that is mainly responsible for the mechanical properties of the skin. The net amount of wound collagen deposition depends on collagen turnover and is a reflection of collagen synthesis minus collagen breakdown. The changes in the diameter of collagen fibrils have also been related to mechanical strength of the skin. Apparently thick collagen fibrils can resist greater tensile strength as opposed to thin ones. “Once the skin is injured, the normal collagen will be replaced by scar collagen and the connective tissue will not regain the original highly organized structure of collagen. Thus, the healing skin is weaker and results in lower tensile strength as opposed to the normal skin.

2.4.2 Basement membranes

BM are delicate structure at the interface between cells and stroma. They contain type IV collagen, laminin, and matrix components

2.4.3 Elastic fiber

Tissue such as blood vessel, lung , uterus, and skin require elasticity for their function. Although tensile strength is provided by members of the collagen family, the ability of these to recoil is provided by elastic fibers. These fibers can stretch to several times their length and then return to their original size after release of the tension. Elastic fibers consist of a central core surrounded by a peripheral microfibrillar network. The central core is made largely of elastin. One third of the residues of elastin are glycin. Mature elastin contains cross-links that regulate its elasticity. The peripheral microfibrillar network that surrounds the

core consists largely of fibrillin. The microfibrils serve as a scaffolding for deposition of elastin and the assembly of elastic fibers.

2.4.4 Structural glycoprotein

Glycoprotein are structurally diverse protein whose major property is their ability to bind with other extracellular matrix component. Glycoprotein consist of fibronectin, osteonectin and tenascin. The fibronectin exists in two major form, plasma fibronectin, and tissue fibronectin. The varied binding properties of fibronectin permit it to connect cell with other components of the extracellular matrix, thereby integrating the tissue into functional unit. The fibronectin is covalently cross-linked with itself, fibrinogen, fibrin or collagen. This cross-linking is probably of great importance in the early phase of wound healing.

2.4.5 Proteoglycans

Proteoglycans are molecules of the extracellular matrix. Proteoglycans consist of glycosaminoglycans linked covalently to a protein core. Proteoglycans are widely distributed in all extracellular metrixs, and they are also found in cell surface and in most biological fluids. They have diverse roles in regulating connective tissue structure and permeability and inmodulating cell differentiation. An organizer of extracellular matrix, these molecules are deposited in the early phase of wound healing, before collagen deposition becomes prominent (Baie and Jude, 2000; Scott, 2001; Cattaruzza and Perris, 2005).

2.5 Diabetes mellitus (Ramamurthy *et al.,* 1998; Labman Schultz and Lehner, 2005; Ahmed and Goldstein, 2006)

DM is a clinically and genetically heterogeneous group of disorders characterized by abnormally high levels of glucose in the blood. The hyperglycemia is due to deficiency of insulin secretion or to resistance of the body's cell to the action of insulin, or to a combination of these. Clinically, the DM can be classified as type I and type II as follows:

2.5.1 Classification of DM

2.5.1.1 Type 1 DM

Caused by β-cell destruct on, often immune meditated, that lead to loss of insulin secretion and absolute insulin deficiency. The etiologic agents that cause the autoimmune process and β-cell destruction are not well established.

2.5.1.2 Type 2 DM

Caused by a combination of genetic and nongenetic factor that result in insulin resistance and insulin deficiency. The specific genes are not known but are under intense investigation. Nongenetic factors include increasing age, high caloric intake, overweight, central adiposity, sedentary lifestyle, and low birth weight.

2.5.2 Diabetic and healing

Patients with diabetes often have wounds that are difficult to heal. The initial barrier to heal is increased blood glucose level, which causes the cell wall to become rigid, impairing blood flow through the critical small vessels at the wound surface and impeding red blood cell permeability and flow. Impairment released of oxygen by hemoglobin results in oxygen and nutrient deficits in the wound. A less optimal immune function also contributes to poor wound healing in patients with diabetes. When blood glucose levels are persistently elevated, chemotaxis and phagocytosis are compromised. Chemotaxis is the process by which white cell are attracted to the site of infection, while phagocytosis is the ingestion of bacteria by white cell. Both processes are important in controlling wound infections. Diabetic infections take a longer time to heal because of delayed macrophage introduction and diminished leukocyte migration, which causes a prolonged inflammatory phase in the wound healing.

The absence or deficiency of insulin DM cause impaired metabolism of carbohydrates, fat and proteins, which are necessary for cellular activities and tissue synthesis in wound healing. Insulin is required for glucose to enter cell as to provide a source of energy for uptake of amino acids to synthesize protein and for inhibition of adipose tissue lipolysis (Ekmektzoglou and Zografos, 2006).

2.6 Skin wound healing in rats

Wound healing is one of the most complex biological events after birth (Gillitzer and Goebeler, 2001). It is a complex process of the replacement of dead tissue by a vital tissue. The response of the body to local injury begins every early in the process of inflammation, and results in repair and regeneration. Regeneration is a replacement of injured tissue by parenchymal cells of the same type, sometimes leaving no residual trace of the previous injury. Repair is a replacement by connective tissue, which in its permanent state constitutes a scar (Menetrey *et al.* 2000). Tissues of mammals consist of a mixture of cells that include three types: permanently dividing – labile cells (e.g. epithelial cells), cells that occasionally go back to the cell cycle – stabile cells (e.g. fibroblasts, satellite cells) and non - dividing cells (striated muscle cells). The rat skin structure is, in many aspects, similar to human skin. The specific structural characteristics may vary depending on the body region (Marcelo *et al*. 2003). The skin of the dorsum of a normal rat is formed by epidermis, dermis and subcutaneous striated muscle. The epithelial appendages are mostly hair follicles and sebaceous glands. However, no sweat glands were observed in rats (Marcelo *et al.* 2003). Hair follicles can play a role in the process of re-epithelialization. If some hair follicles are placed in the incision space, they can serve as other centers of re-epithelialization.

A wound healing of injured dermis runs in three basic phases: inflammation, proliferation and maturation. The phases are not strictly separated from each other, their processes freely blend together. The healing process of injured striated muscle includes three phases: the destruction phase, the repair phase and the remodeling phase (Menetrey *et al.* 2000). During the repair and remodeling phase of healing, there are two concurrent

processes that are at the same time supportive but also competitive with each other (Järvinen *et al*., 2005). The first process is the differentiation of new myofibers from satellite cells. The second one is the formation of the granulation and scar tissue, which serve as a scaffold for regenerating myofibers. This is very similar to the healing of injured dermis.

2.6.1 Incision wound healing process in rats (Vidinsky *et al*., 2006)

In 24 hours, the intense inflammatory reaction was dominant. On the surface, necrosis of skin tissue was situated as a consequence of mechanical damage. The beginning of the formation of the demarcation line was observed under the tissue necrosis. Thus the cellular reaction of inflammatory phase was initiated. The demarcation line consisted of polymorphonuclear leukocytes (PMNL).Macrophages concomitantly invaded the wound area. The epidermis was thickened at its cut edges as a result of mitotic activity of basal cells. The fibrin network filled out the incision space. This network contained blood cells and created a scaffold for migrating fibroblast. The striated muscle showed necrotic myofibers in the deepest part of the wound.

In 48 hrs, it was possible to observe that necrotic debris on the surface was almost removed and the scab was forming. Mainly from the thickened edges of epidermis, keratinocytes migrated beneath the scab, but never bridged the whole incision. The inflammatory infiltrate persisted but the proportion inflammatory cells were changed. PMNL were replaced by tissue macrophages. Fibroblasts were randomly distributed near the incision space. There are syntheses of new extracellar matrix and capillaries. However, the main role in the process of degradation of necrotic myofibers were macrophages which always present in wounds during the first week of healing process.

On day 3, the retirement of inflammatory process was evident. The inflammatory phase was almost completed, PMNL were only randomly dispersed near the incision space and numbers of macrophages were maintained in the wound. The re-epithelialization rapidly continued. The incisions were completely bridged with 3 layers of newly synthesized

epithelial cells. Additionally, the differentiation process of keratinocytes was confirmed by the appearance of keratin layer above the epithelial layers with nuclear cells. The formation of granulation tissue was mainly situated on the bottom of the wounds where moderate number of fibroblast and increasing number of capillaries were presented. The incision space at the layer of dermis and striated muscle contained an extracellar matrix of collagen. The new collagen fibers in the granulation tissue were placed only in focal area.

The healing on 4 days after wounding was characterized by almost total regression of the inflammatory process. This reduction was confirmed by the number of PMNL, the major marker of acute inflammation. Re-epithelialization showed a higher number of newly formed epithelial layers and also the differentiation of nuclear keratinocytes to keratinized cells was continued. At the layer of dermis, fibroblasts were predominantly vertically oriented. However, in granulation tissue, between the edges of striated muscle; they did not exhibit any organization pattern. The angiogenesis reviewed a considerable number of new vessels. Increases in the amount of ECM and collagen fibers were in this organized arrangement placed as small randomly distributed fibers. At the layer of striated muscles, the formation of granulation tissue continued simultaneously with the processes at the layer of dermis. Still the degenerating myofibers were observed. No centrolnucleated cells which was considered as regenerating myofibers was found.

On 5 days of healing wounds showed a completely finished re-epithelialization process. The thickness of epidermis was similar to intact epidermis. This time period showed the proliferative phase with expressive representation of fibroblasts and new vessels. They were mostly situated at the layer of striated muscle in granulation tissue. The granulation tissue also consisted of ECM, with presence of new collagen fibers. They were disorganized and filled out the space between cells in granulation tissue. As compared to earlier period of healing, they presented the major component of ECM. No changes in organization pattern of fibroblast were recorded. The centronucleated cells appeared in the bordering areas of muscle stumps. For these time remodeling and reorganization of ECM and fibroblasts was characterized.

On day 6, still vertically oriented fibroblasts were described. However, on day 7 for the first time fibroblast changed their orientation, they were mostly parallel to the basement membrane. It also reviewed more bundles of collagen fibers and not only fibers sparsely distributed.

On day 7, collagen fibers exhibited more evident organization pattern. There is regress in the number of new capillaries and this decrease could be a result of gradually continuing remodeling phase. Centronucleated cells were situated in areas near the muscle stumps and their number increased only slightly.

In summary, after 7 days of healing, all animals exhibited completely finished re-epithelialization, disappearance of acute inflammatory signs, and reorganization of granulation tissue devoid of cutaneous attachments as well as the presence of regenerating myofibers.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical substances

- Alcohol (SIRIBUNCHA, Thailand)
- Normal saline solution (Klean & Kare, Thailand)
- Streptozotocin (Sigma,USA)
- Formalin (VIDHYASOM CO.,Ltd, Thailand)
- Collagen assay kit (Biocolor, Newtownabbey, Northern Ireland, UK)
- Masson's trichrome stain (Bio-Optica, ITALIA)
- Chloral hydrate (Asia Pacific Specially Limited, Australia)
- Acetic acid (Sigma, USA)
- Xylene (TJ Baker, USA)
- Paraffin (TYCO HEAL THCARE GROUPS LP., USA)
- Hematoxylin (Bio-Optica, ITALIA)
- Eosin stain (Bio-Optica, ITALIA)
- Citrate buffer (Sigma, USA)

3.1.2 Instrument

- Battalian
- Black silk no. 3
- Surgical blade no. 11 (Feather Safety Razor CO.,Ltd., Japan)
- Tensiometer (EZ-Test I 30804100798 Shimadzu Corporation, Japan)
- Vernier caliper
- Skin punch (Tontarra, Germany)

- Cover glass
- Slides
- Rotary microtome (Leica, Vashaw Scientific, Norcross, German)
- Glucometer (Accu-CHEK advantage, USA)
- Glucose oxidase reagent strips (Accu-CHEK advantage, USA)
- Syringes
- Needles
- Cotton pads

3.1.3 Experimental animals

A total of 120 male Spraque-Dawley rats weighing 250-300 grams purchased from The National Laboratory Animals Center, Salaya, Mahidol University, Bangkok were used in this study. The animals were divided into two groups of 60 animals each for non diabetic and diabetic rats models. In each group, the animals were divided into five subgroups of twelve animals each for investigation on day 3 and 7. The rats were caged in the air-conditioned room maintained temperature at 25±1 °C. They were fed with commercial pallet diet CP mice, Pokphan 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 7-day period.

3.1.4 Preparation and extraction of *Malvastrum coronamdelianum.*

Leaves and stem of MC were dried at 60 °C, pulverized and then extracted three times by distilled water. First extraction was made by 80 °C distilled water with a ratio of 100 L / 10 kg. dry weight for 7-8 hrs and then filtered. The residues from first extraction were extracted again by 80 °C distilled water for another two times. Filtrates from three time extraction were mixed together and were dried by spray-dried method (Figure 3.1). A pink-brown powder product from extraction was stored at 5°C. The concentration of MC used in this study were 1%, 5% and 10% (w/v) in freshly prepared distilled water.

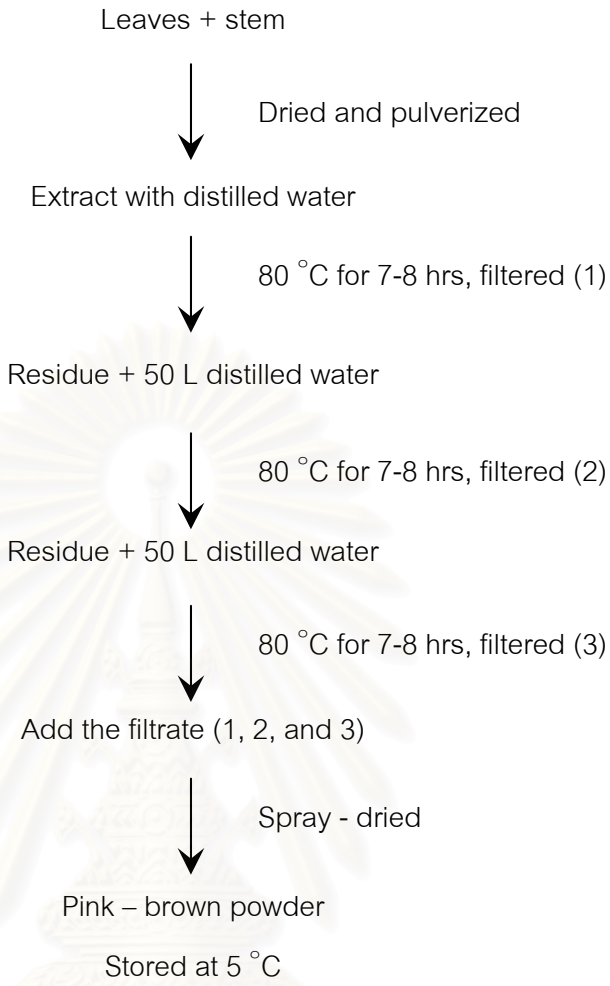

Figure 3.1 Extraction procedure

3.1.5 Microbial limit test

The microbial limit tests are designed to perform the qualitative and quantitative estimations of specific viable microorganism present in MC sample 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 an aseptic petri dish and incubated at 37 °C for 24 hrs. The number of microorganism per ml or gm of sample is counted.

3.2 Methods

3.2.1 Induction of diabetes

Diabetes was induced in rats by a single intravenous injection of streptozotocin (STZ, 50mg/kg. body weight) in 0.1 M citrate buffer, pH 4.0. Fasting blood glucose levels were checked (with glucose oxidase reagent strips) 3 days after STZ injection and animals with glucose levels greater than 200 mg/ml were used for study. Wounds were created on the 7^{th} day after induction of diabetes (Rossini *et al*, 1977; Chithra, Sajithalal and Chandrakasan, 1998).

3.2.2 Wound creation

The animals were anesthetized with chloral hydrate 350 mg/kg body weight, intraperitoneally. The dorsal regions were shaved with a battalion and the surgical area was disinfected with 70% alcohol. A 3-cm linear full thickness incision wound was made on the right side beginning below the inferior edge of the scapula, through the epidermis and dermis to the subcutaneous layer using No.11 sterile surgical scalpel. Each incised wound was closed with 0.5 cm spaced interrupted silk sutures. After recover from anaesthesis, the animals were housed with free access to water and standard laboratory chow. After wounding, 1 ml of the test substances were topically applied to animals once daily. On day 3 and 7 posts wounding the animals were sacrificed with chloral hydrate 400 mg/kg body weight, intraperitoneally. Then the sutures were removed and the tissue was isolated from the healed wound for measurement of tensile strength, collagen content, epidermal thickness and histopathological investigation. The sixty animals were included in this experiment and divided into five groups of twelve animals each. In each group, the animals were divided into five subgroups of twelve animals each for investigate on day 3 and 7 as follows: (Pablo and Mark, 1998) (Figure 3.3)

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

- Group 1, Non diabetic-incision rats without treated
- Group 2, Non diabetic-incision rats treated with normal saline solution
- Group 3, Non diabetic-incision rats treated with 1%MC extract
- Group 4, Non diabetic-incision rats treated with 5%MC extract
- Group 5, Non diabetic-incision rats treated with 10%MC extract

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

- Group 1, DM-incision rats without treated
- Group 2, DM-incision rats treated with normal saline solution
- Group 3, DM-incision rats treated with 1%MC extract
- Group 4, DM-incision rats treated with 5%MC extract
- Group 5, DM-incision rats treated with 10%MC extract

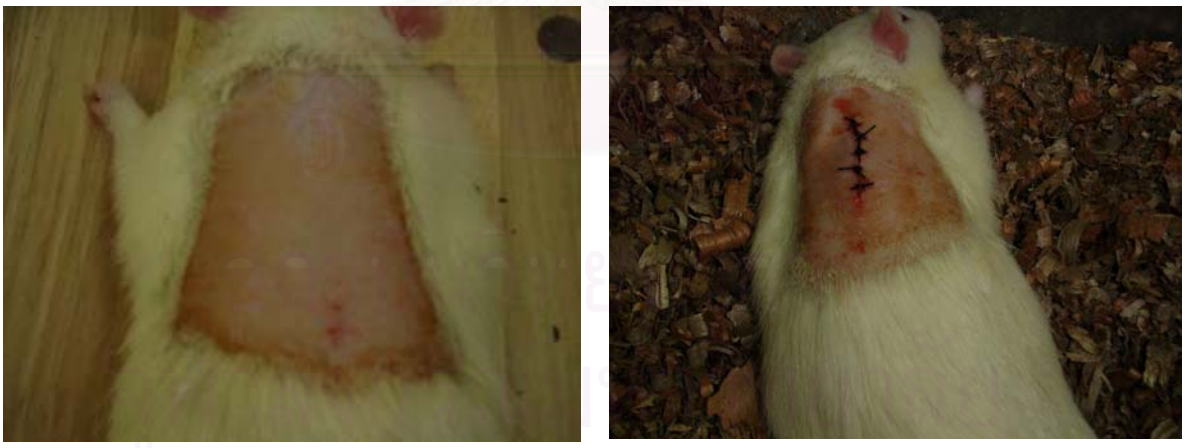

Figure 3.2 The area prepared for wounding

3.2.3 Evaluation of wound healing of incision wound

3.2.3.1 Tensile strength test

The tensile strength of incision wounds were measured on day 3 and 7 post wounding. The sutures were removed and two remaining 0.5 cm sections proximal and distal to the excised section were removed from each animal and immediately test for tensile strength using a skin tensiometer (Figure 3.3, 3.4). The values from the two specimens per animal were averaged for each wound and tensile strength were calculated by the following formula (Baie and Sheikh, 2000; Beloz, Rucinski and Balick, 2003).

$$\text{Tensile strength } (N/cm^2) = \text{Breaking load } (N) \times \text{Area } (cm^2)$$

$$\text{Area } (cm^2) = \text{Thickness } (cm) \times \text{width } (cm)$$

Figure 3.3 The tensiometer

Figure 3.4 The tissue holder

3.2.3.2 Biochemical analysis

For the collagen assay, 5 mm punch biopsy specimens were excised from the shaved back skin and stored at -80 °C. Collagen deposition was estimated by determining the total collagen content of the skin using the Sircol Collagen Assay kit (Biocolor, Newtownabbey, Northern Ireland, UK). The biopsies were homogenized by pepsin, at a ratio of 100 mg pepsin/gm of wet tissue. The required amount of pepsin was dissolved in 0.5 ml of 0.5 M acetic acid and added to the tissue. The sample were carried out for overnight, and 1 ml of Sircol dye reagent was added to each sample, which was then mixed for 30 min. After centrifugation, the pallet was suspended in 1 ml of the alkali reagent included in the kit and assessed colorimetrically at 540 nm by using spectrophotometer. Collagen standard solutions were used to construct a standard curve (Chithra, Sajithalal and Chandrakasan, 1998).

3.2.3.3 Epidermal thickness

The specimen of skin, 0.5x0.5 cm in size, was taken from the middle of wound area. The tissue was preserved in the fresh fixative aqueous 10% neutral buffered solution of formaldehyde for 24 hrs. The Sections of 10 μm thickness were cut and strained with Masson's trichrome dyes. The epidermal thickness was measured from the granular layer to the bottom of the basal layer. The light microscope with x4 and x10 objective lens was used (Pablo and Mark, 1998).

3.2.3.4 Histopathology investigation

The specimen of skin, 0.5x0.5 cm in size, was taken from the middle of wound area. The tissue was preserved in the fresh fixative aqueous 10% neutral buffered solution of formaldehyde 24 hrs. The sections of 10 μm thickness were cut and strained with hematoxylin and eosin dyes. The light microscope with x4 and x10 objective lens were used (Pablo and Mark, 1998).

3.2.4 Antibacterial activity of *Malvastrum coromandelianum* (*in vitro* study).

Determination of the different concentrations of MC against *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Escherichia coli* by Filter paper disk- agar diffusion method.

3.2.4.1 Method

The nutrient medium used was Mueller-Hinton Agar. It was prepared by mixing 38 gram of Mueller-Hinton Agar with 1L of water and autoclaved at 121 °C for 30 min (Deewees, Poupard, and Morton, 1970; Lorian and Sttrauss, 1966). Media was allowed to cool to about 55 °C, and then poured into plates. Plates were let cool overnight. Colonies of *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Escherichia coli* were touched from assigned plate culture and dissolved in 0.87% normal saline solution 5 ml until they match the turbidity of McFarland standard no. 5. Using a sterile cotton swab, inoculate on a nutrient agar plate. A 6 mm filter paper disc was impregnated with normal saline solution and the different concentrations of MC (1%, 5% and 10%), were gently pressed with flame and cool forceps on to the surface of the agar. The plates were incubated at 37 °C for 24 hrs. After incubation, the plates were measured the zone of inhibition by using vernier caliper (Lorian and Strauss, 1966; Dewees, Poupard and Morton, 1970; Saeed *et al,* 2006).

3.3 Data analysis

Results were presented as mean $\pm$ S.E.M. The differences among experimental groups were compared by ANOVA followed by Duncan post hoc test and were considered statistically significant when P was less than 0.05.

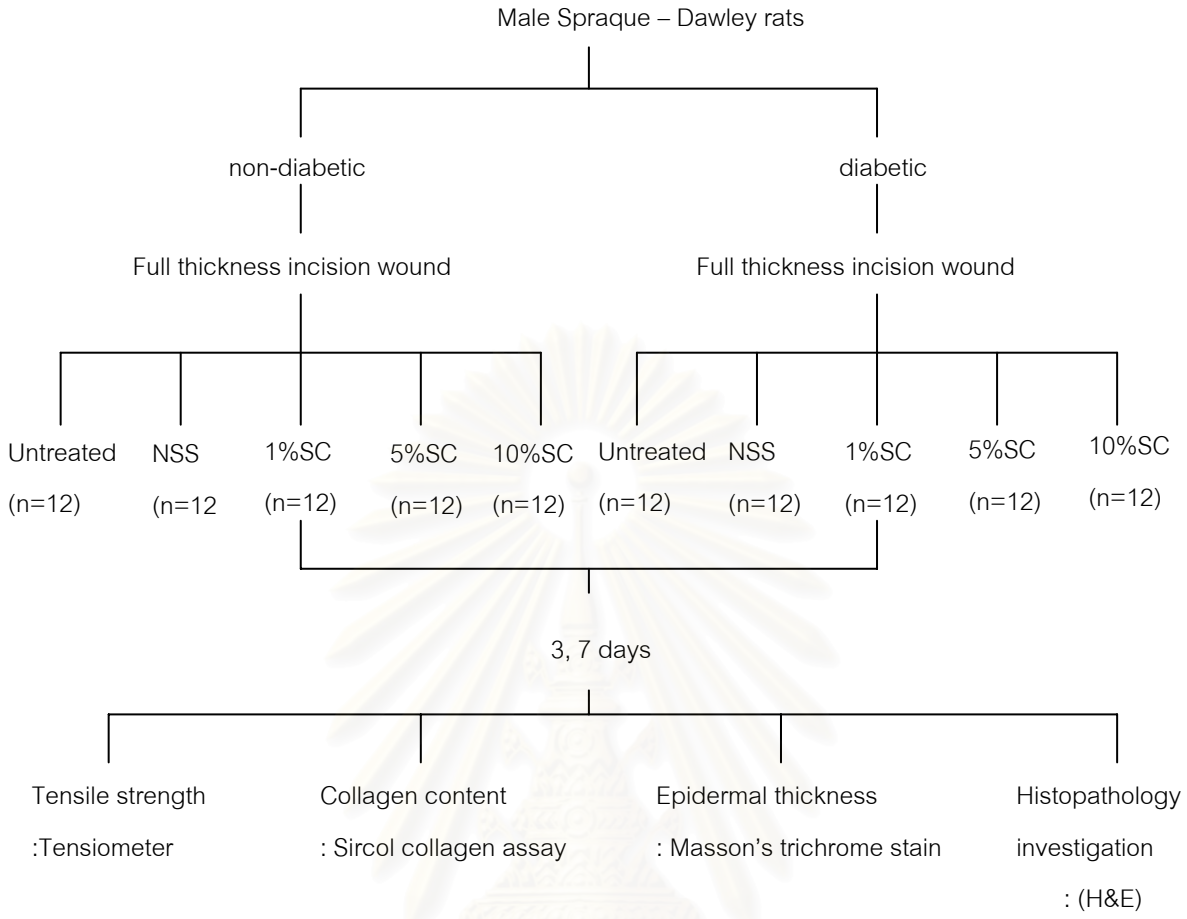

Figure 3.5: Diagram of experimental animal group

CHAPTER IV

RESULTS

4.1 Effects of the *Malvastrum coromandelianum* extract on wound healing in non-diabetic rats.

4.1.1 Tensile strength

Healing of incision wound was evaluated by a measurement of tensile strength on day 3 post wound. The tensile strength of untreated animals was not significantly different from those found in NSS – treated group. In comparison to untreated, and NSS – treated groups, tensile strength in animal groups treated with 1% of MC extract was significantly increased for 5.80 ± 0.24 N/cm^2 whereas it was 3.87 ± 0.38 N/cm^2 and 3.70 ± 0.26 N/cm^2 in untreated and NSS-treated groups, respectively (Figure 4.1). Tensile strength of 5% and 10% MC groups were not significantly different from untreated or NSS-treated group.

On day 7 post wound, the tensile strength of untreated animals (24.63 ± 3.18 N/cm^2) was not significantly different from those found in NSS – treated group (24.34 ± 2.19 N/cm^2) or 1%, 5% and 10% of different concentration from MC extract. However, none of them was significantly different from those of NSS - treated groups (Figure 4.1).

4.1.2 Epidermal thickness

At day 3 post wounding, the epidermal thickness were measured by Masson's trichrome-stained. The epidermal thickness of untreated animals was not significantly different from those found in NSS – treated group. In comparison to untreated, and NSS – treated groups, epidermal thickness in animal groups treated with 1% of MC extract was significantly increased for 95 ± 8.06 μm whereas it was 69.25 ± 3.11μm and 68.46 ± 7.05 μm in untreated and NSS-treated groups, respectively. Epidermal thickness of 5% and 10% MC groups was not significantly different from untreated or NSS-treated group.

At day 7 post wounding, the epidermal thickness of untreated group (97.5 ± 13.76 μm) was not significantly different from those found in NSS – treated group (100.83 ± 7.68 μm) or 1%, 5% and 10% of different concentration from MC extract. However, none of them was significantly different from those of NSS - treated groups (Figure 4.2).

4.1.3 Collagen content

At day 3 post wounding, the wound collagen content was determinated by Sircol collagen assay kit. Values are presented as micrograms total collagen (Types I–V). Wounds receiving 1%, 5% and 10% of different concentration from MC extract were no significantly different from those of untreated and NSS - treated groups.

At day 7 post wounding, the wound collagen content was determinated by Sircol collagen assay kit. Values are presented as micrograms total collagen (Types I–V). Wounds receiving 1%, 5% and 10% of different concentration from MC extract were not significantly different from those of untreated and NSS - treated groups (Figure 4.3).

4.1.4 Histopathological observation

Histopathological evaluation of wound healing in this study was examined at the end of experiment on day 3 and 7 in non-diabetic rats.

On day 3, the wound of all groups showed a re-epithelialization. However, the wound treated with 1% of MC is showed a formation of granulation and hair follicles in the edge of wound (Figure 4.4).

On day 7, the wound in untreated, NSS–treaded and different concentration of MC (1%, 5% and 10%) group showed that the healing have not different (Figure 4.5).

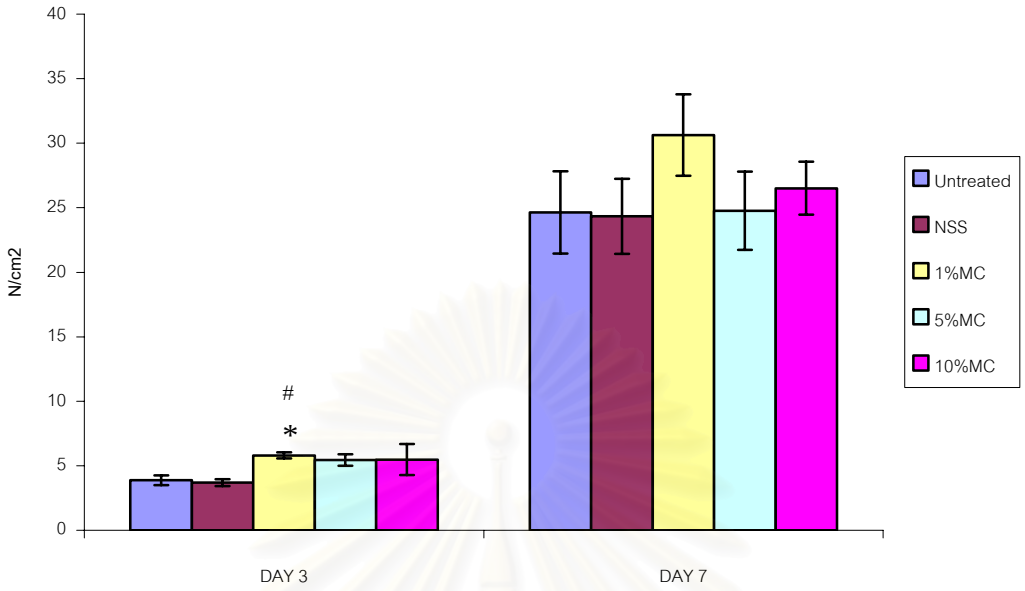

Figure 4.1 Bar graph showing the Mean ± S.E.M of tensile strength on day 3 and 7 in non-diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

* Significant difference compared to untreated ($P \leq 0.05$)

Significant difference compared to normal saline-treated ($P \leq 0.05$)

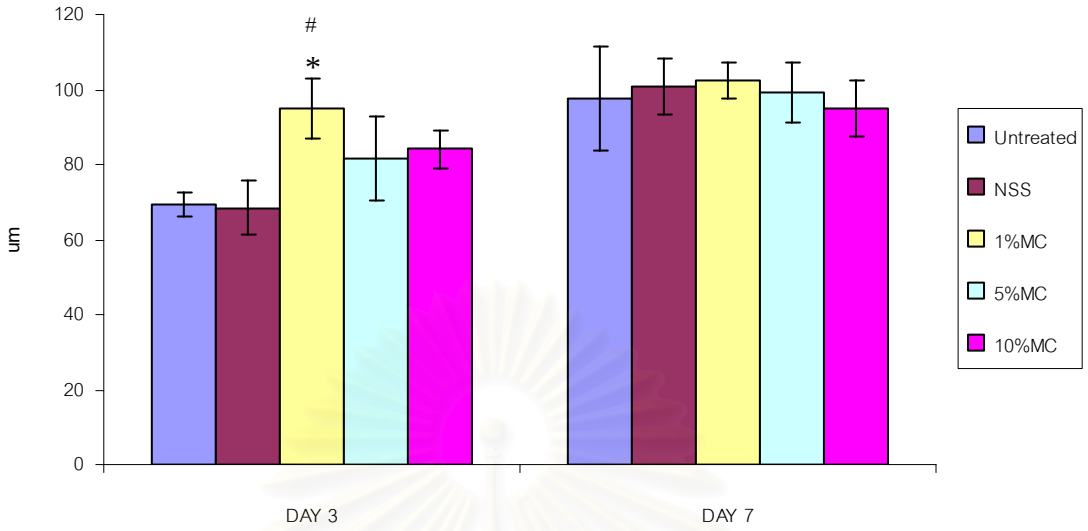

Figure 4.2 Bar graph showing the Mean ± S.E.M of epidermal thickness (stain with Masson's trichrome) on day 3 and 7 in non-diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

* Significant difference compared to untreated ($P \leq 0.05$)

Significant difference compared to normal saline-treated ($P \leq 0.05$)

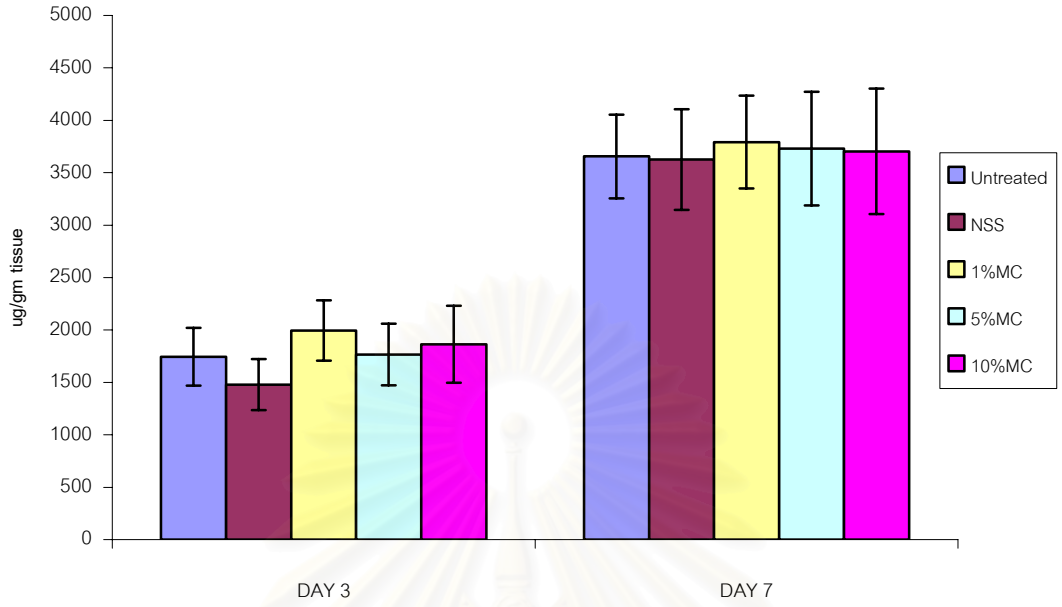

Figure 4.3 Bar graph showing the Mean ± S.E.M of collagen content on day 3 and 7 in non-diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

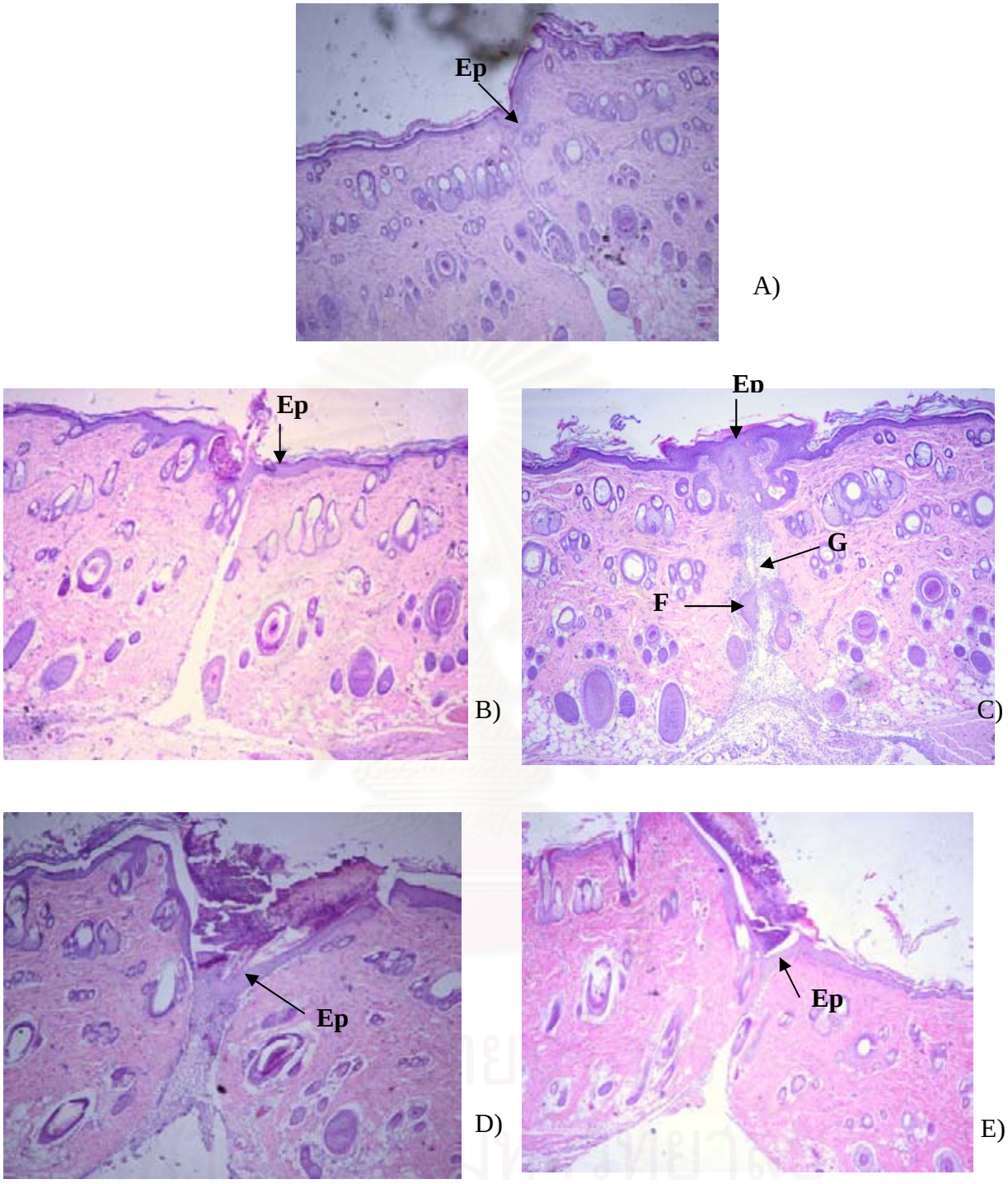

Figure 4.4 Histological change of skin section stain with Heamatoxylin – eosin, at day 3 in non- diabetic group post wounding of A) untreated, B) NSS-treated, C) 1% MC, D) 5% MC, E) 10%MC. Ep=Epidermis, F=Hair follicle, G=Granulation tissue.

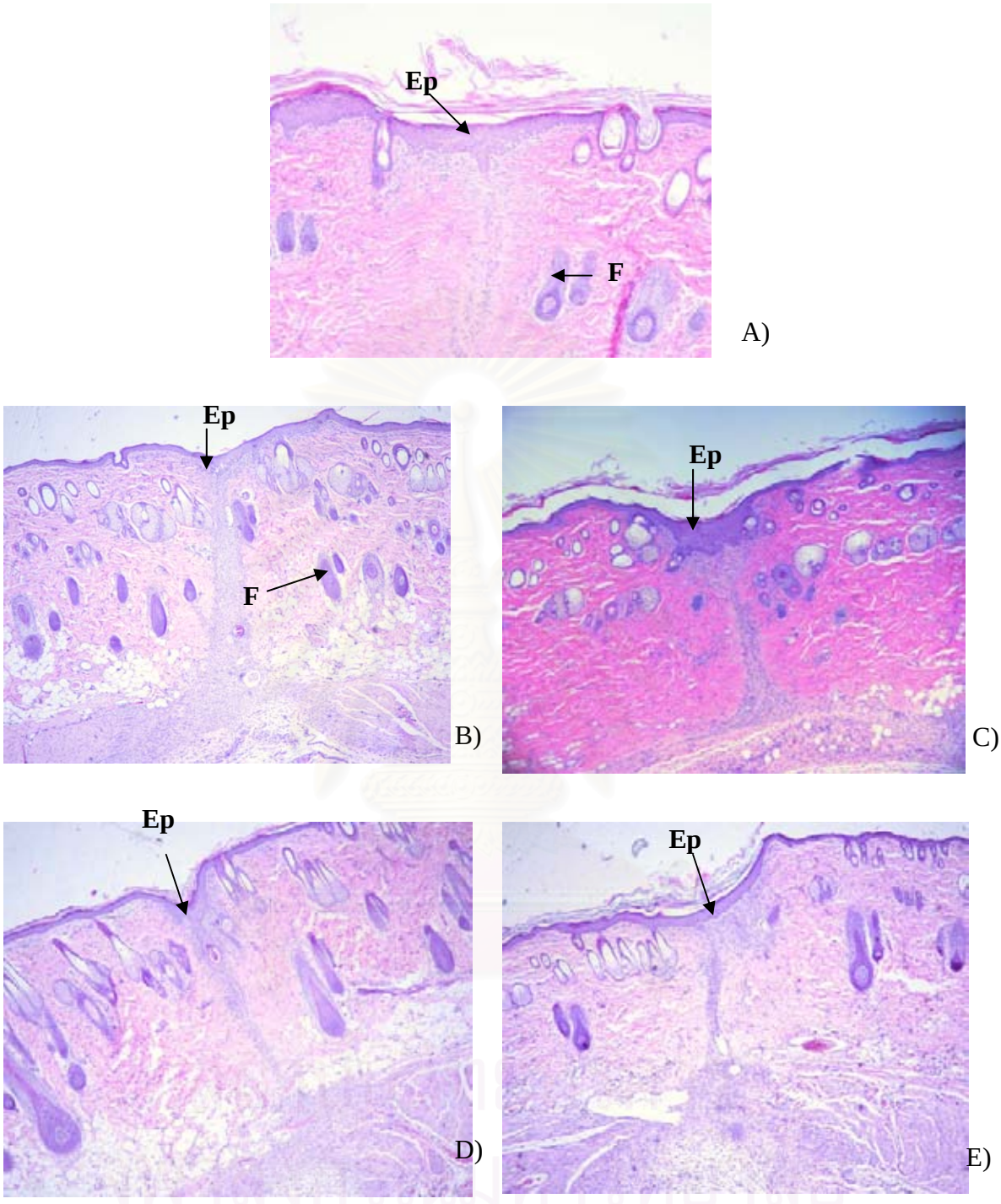

Figure 4.5 Histological change of skin section stain with Heamatoxylin – eosin, at day 7 in non-diabetic group post wounding of A) untreated, B) NSS-treated, C) 1% MC, D) 5% MC, E) 10%MC.Ep=Epidermis, F=Hair follicle.

4.2 Effects of the *Malvastrum coromandelianum* extract on wound healing in diabetic rat.

4.2.1 Tensile strength

Healing of incision wound was evaluated by measurement of tensile strength on day 3 post wound. The tensile strength of untreated animals (3.39 ± 0.64 N/cm^2) was not significantly different from those found in NSS – treated group (3.26 ± 0.51 N/cm^2) or 1%, 5% and 10% of different concentration from MC extract.

On day 7 post wound, the tensile strength of untreated animals was not significantly different from those found in NSS – treated group. In comparison to control, and NSS – treated groups, tensile strength in animal groups treated with 1% of MC extract was significantly increased to 13.98 ± 1.70 N/cm^2 whereas they were 8.78 ± 1.40 N/cm^2 and 8.86 ± 0.81 N/cm^2 in untreated and NSS-treated groups, respectively. Increment in tensile strength was also observed in 5% and 10% MC groups but they were not significantly different from untreated or NSS-treated group (Figure 4.6).

4.2.2 Epidermal thickness

At day 3 post wounding, the epidermal thickness were measured by Masson's trichrome-stained. The group of untreated animals (49.16 ± 8.89 μm) was not significantly different from those found in NSS – treated group (52.50 ± 4.95 μm) or 1%, 5% and 10% of different concentration from MC extract. However, none of them was significantly different from those of NSS - treated groups.

At day 7 post wounding, the epidermal thickness of untreated animals was not significantly different from those found in NSS – treated group. In comparison to control, and NSS – treated groups, epidermal thickness in animal groups treated with 1% of MC extract was significantly increased to 98.33 ± 12.24 μm whereas they were 65.83 ± 8.89 μm and 67.5 ± 8.34 μm in untreated and NSS-treated groups, respectively. Increment in epidermal

thickness was also observed in 5% and 10% MC groups but they were not significantly different from control or NSS-treated group (Figure 4.7).

4.2.3 Collagen content

At day 3 post wounding, the collagen content of untreated group (1276.21 ± 179.14 μg/g) was not significantly different from those found in NSS – treated group (1223.79 ± 225.46 μg/gm) or 1%, 5% and 10% of different concentration from MC extract. However, none of them was significantly different from those of NSS - treated groups.

At day 7 post wounding, the collagen content of untreated group (2359.84 ± 445.73 μg/gm) was not significantly different from those found in NSS – treated group (2272.14 ± 495.99 μg/gm) or 1%, 5% and 10% of different concentration from MC extract. However, none of them was significantly different from those of NSS - treated groups (Figure 4.8).

4.2.4 Histopathological observation

Histopathological evaluation of wound healing in this study was examined at the end of experiment on day 3 and 7 in diabetic rats.

On day 3, the wound of all groups showed a re-epithelialization cells. The healing is poorer than non diabetic rats (Figure 4.9).

On day 7, the wound were completely closed. The wound in untreated and NSS–treated have many of inflammatory cells while the wound-treated with 1%MC has no sign of acute inflammation (Figure 4.10).

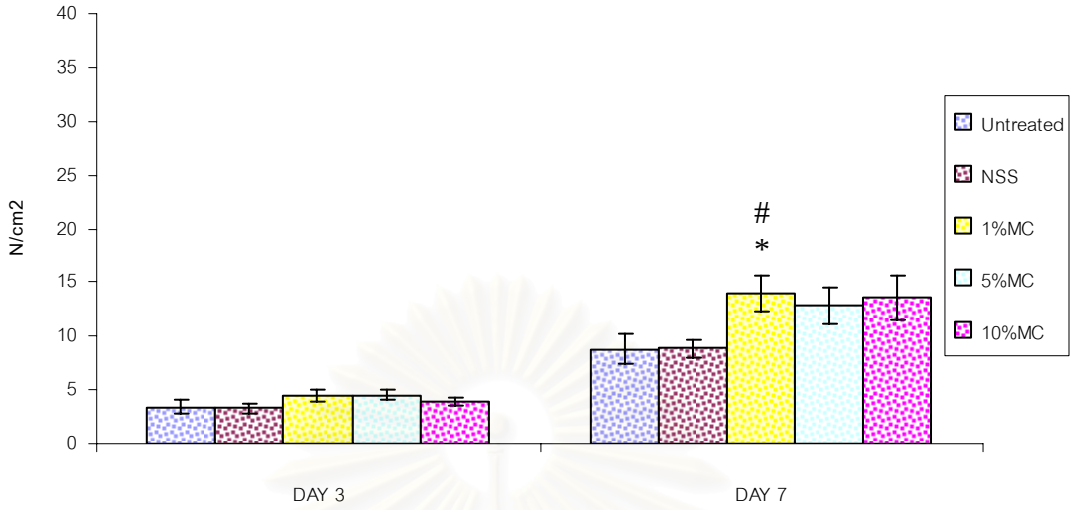

Figure 4.6 Bar graph showing the Mean ± S.E.M of tensile strength on day 3 and 7 in diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

* Significant difference compared to untreated ($P \leq 0.05$)

Significant difference compared to normal saline-treated ($P \leq 0.05$)

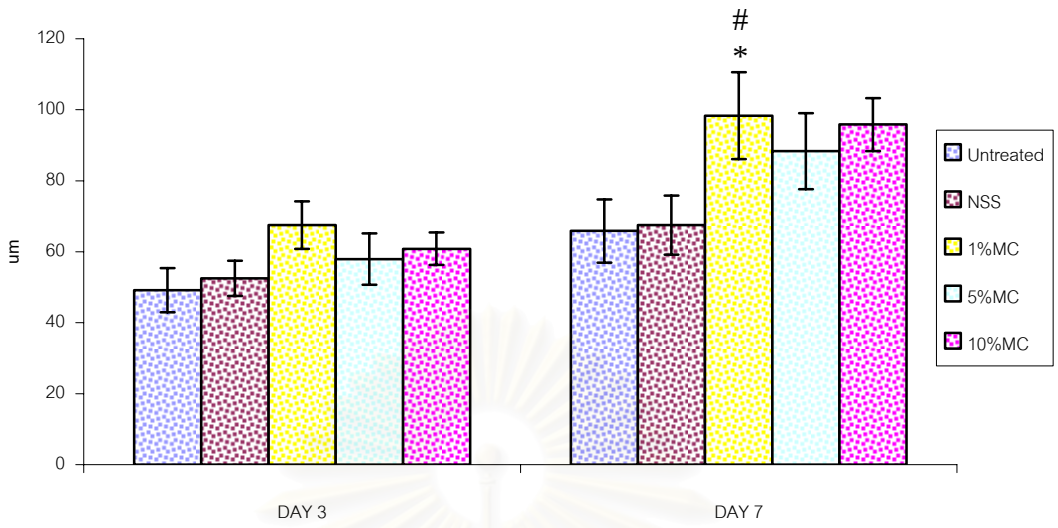

Figure 4.7 Bar graph showing the Mean ± S.E.M of epidermal thickness (stain with Masson's trichrome) on day 3 and 7 in diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

* Significant difference compared to untreated ($P \leq 0.05$)

Significant difference compared to normal saline-treated ($P \leq 0.05$)

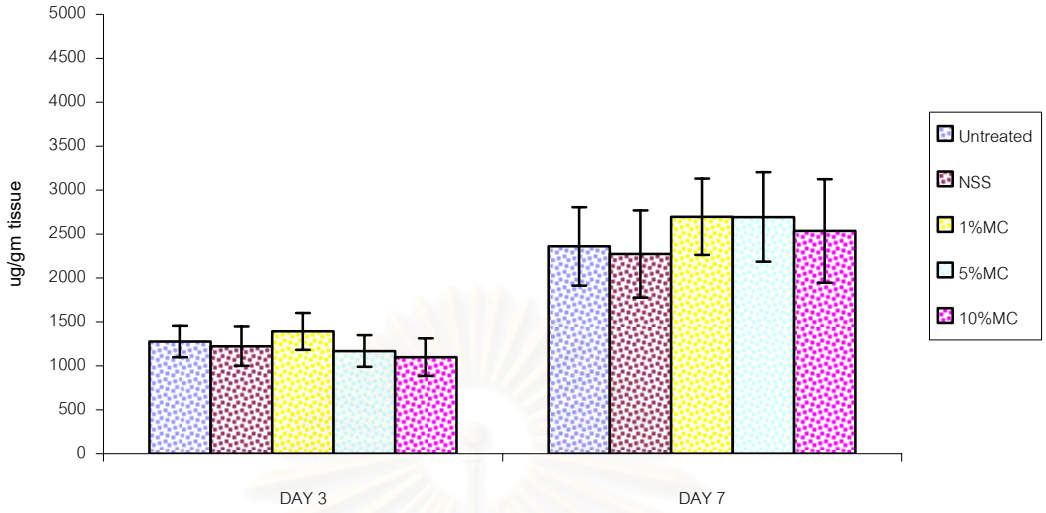

Figure 4.8 Bar graph showing the Mean ± S.E.M of collagen content on day 3 and 7 in diabetic rats.

Untreated; incision wound-without treated

NSS; incision wound-normal saline treated

1% MC; incision wound-1%MC treated

5%MC; incision wound-5%MC treated

10%MC; incision wound-10%MC treated

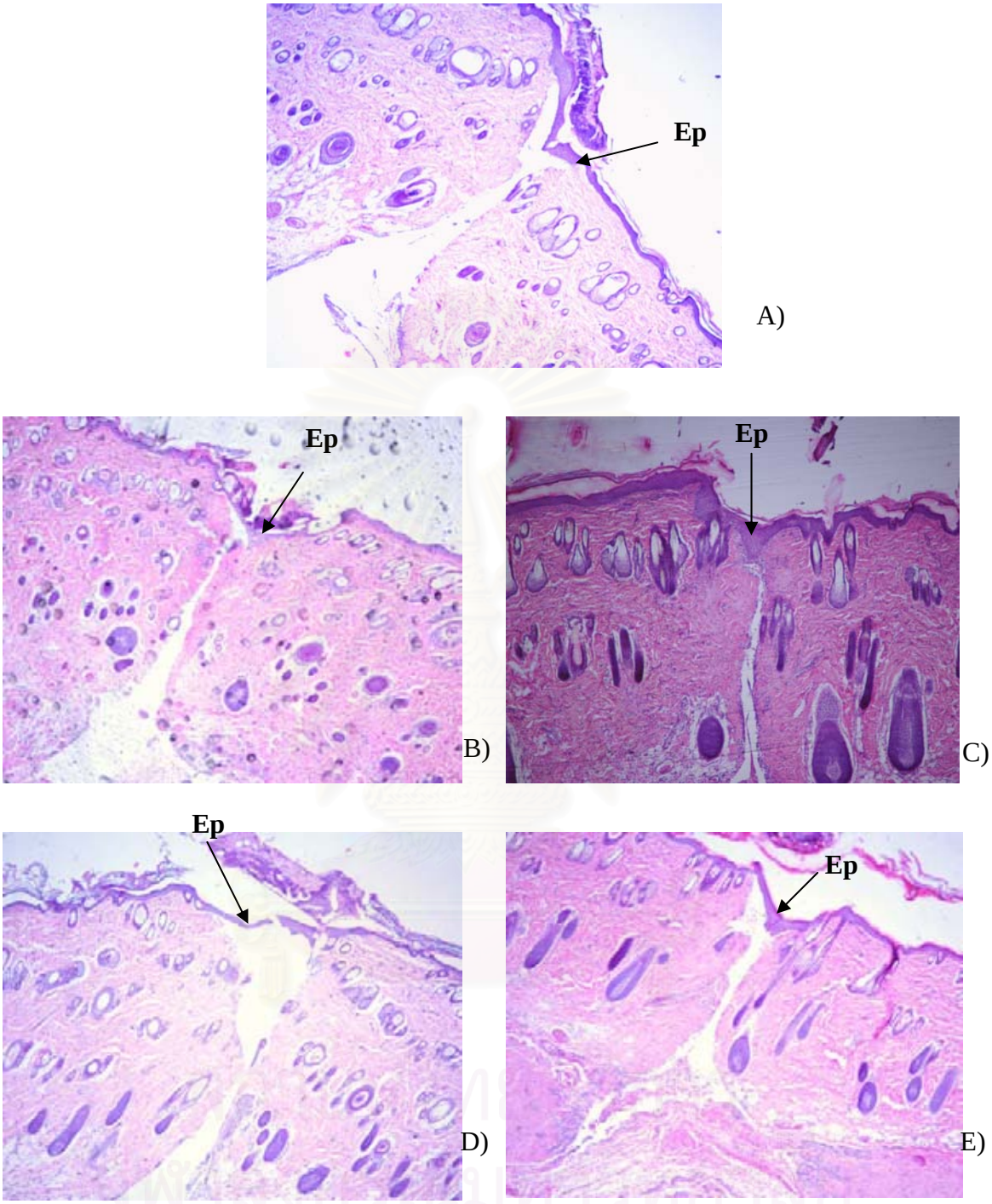

Figure 4.9 Histological change of skin section stain with Heamatoxylin-eosin, at day 3 in diabetic group post wounding of A) untreated, B) NSS-treated, C) 1% MC, D) 5% MC, E) 10%MC. Ep=Epidermis.

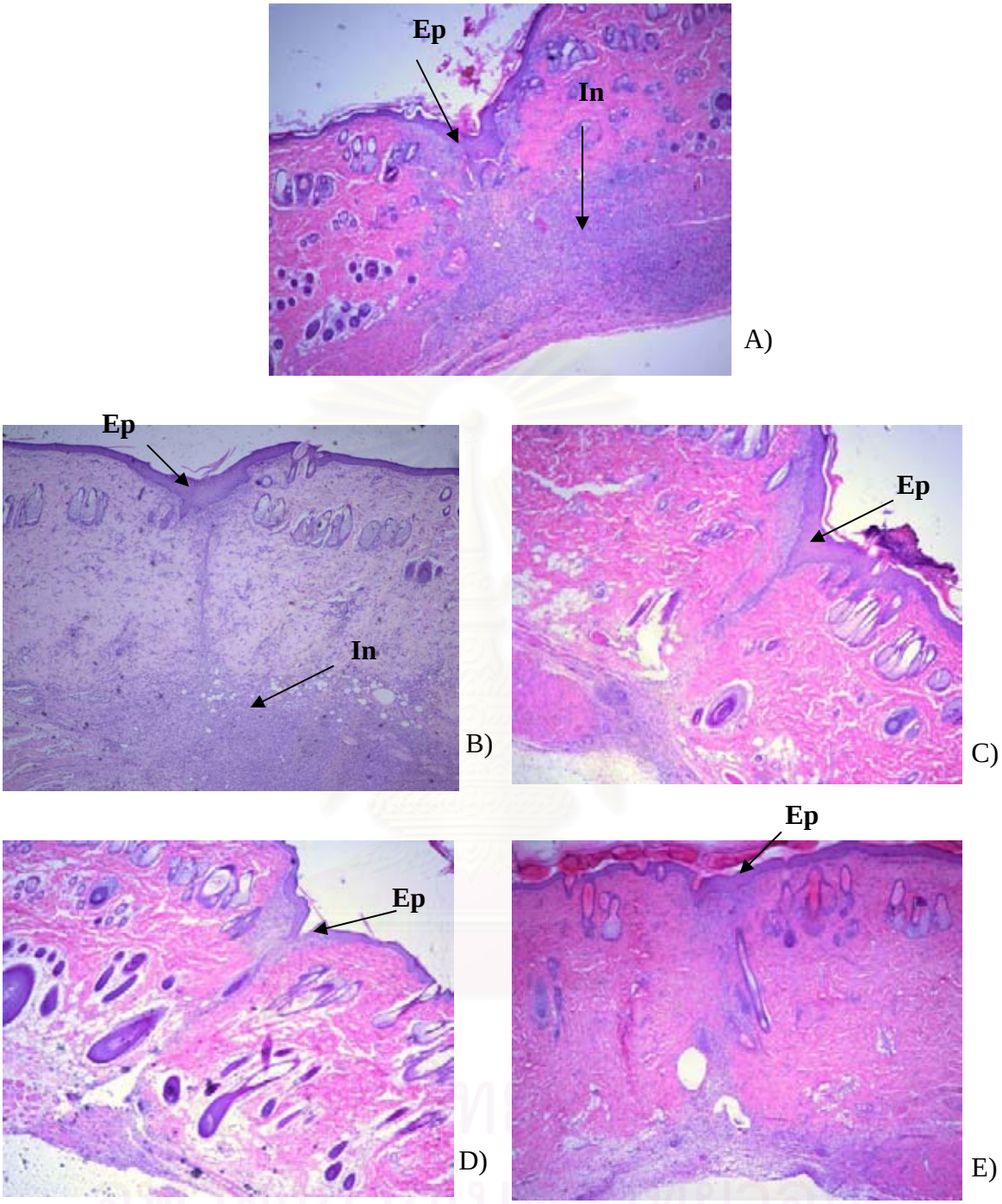

Figure 4.10 Histological change of skin section stain with Heamatoxylin-eosin, at day 7 in diabetic group post wounding of A) untreated, B) NSS-treated, C) 1% MC, D) 5% MC, E)10%MC.Ep=Epidermis, In=Inflammatory cell.

4.3 Antibacterial activity of *Malvastrum coromandelianum*

The different concentration of MC (1%, 5% and 10%) can not inhibit against *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginnosa* and *Escherichia coli*.

Group	Inhibition zone (mm^2)				
	NSS	1% MC	5% MC	10% MC	Povidone-iodine
Staphylococcus aureus	0	0	0	0	136
Streptococcus pyogens	0	0	0	0	85.19
Pseudomonas aeruginosa	0	0	0	0	38.34
Escherichia coli	0	0	0	0	59.17

Table 4.1 Area of Inhibition zone (mm^2) result present as mean.

CHAPTER V

DISCUSSION

Medicinal plants have long been integrated into primary health care system of Thailand. Various plants such as *Centella asiatica, Aloe vera* and *Durio zibethinus* are recommended to be grown and used in households as well as in the hospitals for wound healing (Suguna, Savakumar and Chandrakasan,1996; Chithra and Sajithlal, 1997; Lipipun, Nantawanit and Pongsamart, 2002). Crude extract of *Centella asiatica* has been shown to increase the tensile strength of incision wound (Suguna, Savakumar and Chandrakasan, 1996). Antidiabetic as well as wound healing properties of *Aloe Vera* have been reported (Chithra and Sajithlal, 1997). Similar to those mentioned above, we found that the crude extract of MC exerted beneficial effect on incision wound healing in both non-diabetic and STZ-induced diabetic rats.

Cutaneous wound repair *in vivo* is a dynamic, complex process involves vascular response characterized by blood coagulation and hemostasis as well as cellular events including infiltration of leukocytes with varied functions of antimicrobial and release of cytokines to initiate the proliferate response for wound repair. During the proliferative phase, there is a formation of the epithelium to cover the wound surface with concomitant growth of granulation tissue to fill the wound space. Granulation tissue formation involves proliferation of fibroblast, deposition of collagen and other extracellular matrixes and angiogenesis. Once the new tissue within the wound is formed, the remodeling phase begins to restore structural integrity and functional competence (Midwood, Williams and Schwarzbauer, 2004; Li, Chen, and Kirsner, 2007).

In the present studies, rate of wound healing of incision wound in both non-diabetic and diabetic rats was assessed by the measurement of tensile strength, collagen content and thickness of the epithelium. In non-diabetic rats, on day 3 post wounding, we found that tensile strength of 1% but not 5% and 10% MC treated group was significantly

higher than those of untreated and NSS treated groups, indicating that healing of incision wound was promoted by topical application of 1% MC extract. Strength of healed wound has been claimed to depend on the deposition of adequate extracellular matrix which is composed of five major components namely, collagen, elastic fiber, basement membranes, glycoprotein and proteoglycan (Rubin and Farber, 2004). As collagen content which is often claimed to be closely interlinked to tensile strength was not significantly increased in the present studies, it is likely that beneficial effects of MC extract on wound healing should be explained by its effect on some other components than stimulation of collagen synthesis. Accordingly, histopathological evaluation did show accumulation of macrophage and granulation tissue in wound space of 1%MC- treated rats, signifying the beginning of proliferative phase. In addition, epidermal thickness of non-diabetic rats treated with 1% of MC was found to be significantly higher than those of untreated and NSS treated groups. Re-epithelialization is apparently governed by the release of epidermal growth factor (EGF) released from macrophages and platelets (Enoch and Leaper, 2005). Therefore it is suggestive that MC extract might stimulate the release of such growth factors or else it contains some growth factor-like substances. Closed wound surface is necessary to create a hypoxia environment in which angiogenesis is facilitated and adequate blood supply is essential to neo-vascularization (Strodtbeck, 2001). 1%MC was reported to significantly increase blood supply to wound area (Sookkul, 2007). Therefore, the increase in tensile strength observed in non-diabetic rats could be attributable to MC's effects on some other components than collagen of extracellular matrix, increase of re-epithelialization and probably an increase in blood supply to the wound area.

On day 7 post wounding, no difference in tensile strength, epidermal thickness and collagen content was noted among MC treated and untreated or NSS treated groups. Together with the finding in histology that fibroblast started to rearrange, thus remodeling in which granulation tissue would be converted to a stable extracellular matrix (Strodtbeck, 2001) has initiated.

Patients with DM exhibited delayed wound closure. Complicated diabetes, especially poorly controlled DM can induce delay wound close and often causing infection, vascular complication with insufficiency, connective tissue abnormality, reduced biosynthesis and/or accelerated degradation of collagen (Chithra, Sajithal and Chandrakasan, 1997). Similarly, in comparison to non-diabetic rats, STZ-induced diabetic rats demonstrated a delay in wound healing. On day 3, whereas the beneficial effect of 1% MC was demonstrated in non-diabetic rats, the tensile strength, epidermal thickness and collagen content of MC-treated diabetic rats were not significantly different from untreated or NSS treated groups. However, the effects of 1% MC extract were clearly demonstrated in tensile strength as well as in epidermal thickness of diabetic rats on day 7 post wounding. In line with the results in non-diabetic rats, 1% MC extract, though, significantly increased the tensile strength, had no effect on collagen amount of diabetic rats. Therefore, lack of effect on collagen of MC extract was then confirmed.

On day 7 post wounding, rather many inflammatory cells were histologically noted in untreated and NSS-treated groups, suggesting that inflammatory phase seemed to be prolonged in diabetic rat. The diabetic wound appears to be stuck in the inflammatory phase and takes a long time to heal as previously reported (Falanga, 2005). Topical application of MC extract tends to limit inflammation as fewer inflammatory cells, less infiltrate of macrophage and fibroblast were observed in 1%MC treated group when compared to untreated and NSS-treated groups. Anti-inflammatory effect of MC extract recently reported by Reddy, Venkatesh and Suresh (2001) should be a possible explanation for the results observed.

Antimicrobial activity of MC against *Staphylococcus aureus*, *Streptococcus pyogens, Pseudomonas aeruginosa* and *Escherichia coli* at the minimum inhibitory concentration of 25% w/v has been reported by Pongpech *et al*, (2005). However, we found that MC extract at the concentration of 1%, 5% and 10% used in the present studies exhibited no antibacterial activity. Therefore, it is unlikely that antimicrobial activity was involved in the wound healing observed in MC extract-treated rats.

CHAPTER VI

CONCLUSION

In conclusion, the present studies have demonstrated the wound healing effect of the extract of MC on incision wound of both non-diabetic and diabetic rats, though with a slower rate. An increase in epidermal thickness and/or some other effects on other components than collagen of extracellular matrix exerted by 1%MC extract seem to underlie an increase in tensile strength observed. Further investigation on the active constituents as well as possible effect on other elements of wound healing should be carried out.

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APPENDICES

Effect of MC on incision wound healing in non-diabetic rats

Group	Tensile strength (N/cm^2)	
	On day 3	On day 7
Untreated(n=6)	3.8742 ± 0.37	24.6300 ± 3.17
NSS(n=6)	3.7007 ± 0.26	24.3308 ± 2.90
1%MC(n=6)	5.8042 ± 0.24 * #	30.6320 ± 3.16
5%MC(n=6)	5.4408 ± 0.44	24.7575 ± 3.04
10%MC(n=6)	5.4867 ± 1.20	25.5058 ± 2.04

Table 1 : Tensile strength in non-diabetic rats (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$)

Group	Epidermal thickness (μm)	
	On day 3	On day 7
Untreated(n=6)	69.25 ± 3.11	97.5 ± 13.76
NSS(n=6)	68.45 ± 7.05	100.83 ± 7.68
1%MC(n=6)	95.00 ± 8.06 * #	102.50 ± 4.96
5%MC(n=6)	81.67± 11.01	99.16 ± 8.21
10%MC(n=6)	84.17 ± 5.07	95.0 ± 7.30

Table 2 : Epidermal thickness in non-diabetic rats (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

Group	Collagen content (μg/gm tissue)	
	On day 3	On day 7
Untreated(n=6)	1745.177 ± 275.72	3655.83 ± 400.04
NSS(n=6)	1478.42 ± 243.74	3625.60 ± 476.46
1%MC(n=6)	1995.12 ± 287.74	3792.603 ± 442.91
5%MC(n=6)	1765.33 ± 294.74	3730.49 ± 540.77
10%MC(n=6)	1863.64 ± 367.71	3704.08 ± 599.48

Table 3 : Collagen content in non-diabetic rats (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

Effect of MC on incision wound healing in diabetic rats

Group	Tensile strength (N/cm^2.)	
	On day 3	On day 7
Untreated(n=6)	3.39 ± 0.64	8.78 ± 1.4
NSS(n=6)	3.26 ± 0.51	8.86 ± 0.81
1%MC(n=6)	4.46 ± 0.49	13.98 ± 1.7 * #
5%MC(n=6)	4.54 ± 0.52	12.86 ± 1.67
10%MC(n=6)	3.95 ± 0.37	13.57 ± 2.09

Table 4 : Tensile strength in diabetic group (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

Group	Epidermal thickness (μm)	
	On day 3	On day 7
Untreated(n=6)	49.16 ± 6.24	65.83 ± 8.89
NSS(n=6)	52.50 ± 4.95	67.5 ± 8.34
1%MC(n=6)	67.5 ± 6.67	98.33 ± 12.24 * #
5%MC(n=6)	57.91 ± 7.2	88.33 ± 10.69
10%MC(n=6)	60.83 ± 4.55	95.83 ± 7.46

Table 5 : Epidermal thickness in diabetic group (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

Group	Collagen content (μg/gm tissue)	
	On day 3	On day 7
Untreated(n=6)	1276.21 ± 179.14	2359.84 ± 445.73
NSS(n=6)	1223.79 ± 225.46	2272.14 ± 495.99
1%MC(n=6)	1392.45 ± 208.20	2696.62 ± 434.62
5%MC(n=6)	1168.88 ± 180.27	2694.22 ± 509.33
10%MC(n=6)	1099.26 ± 213.51	2535.17 ± 589.19

Table 6 : Collagen content in diabetic group (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

Group	Blood glucose level (mg/dl)	
	On day 3	On day 7
Untreated(n=6)	333.33±47.99	355.83±42.89
NSS(n=6)	315.17±30.81	336.16±32.89
1%MC(n=6)	401.00±31.98	362.67±35.86
5%MC(n=6)	344.50±21.40	304.17±58.24
10%MC(n=6)	340.16±43.46	347.83±40.77

Table 7 : Blood glucose level in diabetic group (Mean ± S.E.M.) Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 was considered as statistical significant. * Significant difference compared to Untreated ($P \leq 0.05$). # Significant difference compared to Normal saline-treated ($P \leq 0.05$).

BIOGRAPHY

Miss Ratchaneekorn Panitchakorn was born on November 26, 1980 in Nakornsitammarat, Thailand. She was graduated with Bachelor degree of Science in Physical Therapy in 2002 from Faculty of Health Science, Thammasat University.