นิพนธ์ต้นฉบับ • Original Article

ผลของใบบัวบกและเปลือกมังคุดต่อกระบวนการหายของแผลในหนูที่ถูกเหนี่ยวนำให้เกิดเบาหวาน

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Effects of *Centella Asiatica Linn.* Leaves and *Garcinia Mangostana Linn.* hull on the Healing of Dermal Wounds in Diabetic Rats

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หลักการและเหตุผล: เบาหวานเป็นสาเหตุสำคัญอย่างหนึ่งที่มีผลกระทบต่อกระบวนการหายของแผล ซึ่งแผลเบาหวานจะเกิดเป็นแผลเรื้อรังได้ง่าย บัวบกและเปลือกมังคุดเป็นสมุนไพรไทยที่มีการนำไปใช้ในการรักษาแผลอย่างแพร่หลาย แต่ยังไม่เคยมีการนำมาใช้ในการรักษาแผลเบาหวาน

วัตถุประสงค์: เพื่อศึกษาผลของการใช้บัวบกและเปลือกมังคุดในการรักษาแผลเบาหวาน

วิธีการวิจัย: ใช้หนูขาวพันธุ์ Spraque-Dawley เพศผู้ (64 ตัว) แบ่งออกเป็น 2 กลุ่ม คือ หนูปกติและหนูที่ถูกเหนี่ยวนำให้เกิดเบาหวาน แต่ละกลุ่มแบ่งออกเป็น 4 กลุ่ม ทำให้เกิดแผล จากนั้นรักษาด้วย ยาหลอก บัวบก เปลือกมังคุด และ neomycin

ผลการวิจัย: จากการศึกษาพบว่า หนูกลุ่มที่รักษาด้วยบัวบกและเปลือกมังคุด มีการสร้างส่วนประกอบภายในเซลล์ให้สมบูรณ์ได้เร็วกว่า และอัตราการหดตัวของแผลมีมากกว่า เมื่อเปรียบเทียบกับกลุ่มที่ไม่ได้รักษาด้วยบัวบกและเปลือกมังคุด

สรุป: จากผลการศึกษาที่ได้ ใช้เป็นเครื่องยืนยันอย่างหนึ่งถึงคุณสมบัติและประโยชน์ที่มีของสมุนไพรทั้งสองชนิด ในอนาคตควรจะมีการศึกษาโดยใช้สารสำคัญ ซึ่งเป็นส่วนประกอบที่สกัดจากสมุนไพร เพื่อให้ทราบถึงผลของสารสำคัญต่อกลไกระบวนการหายของแผลต่อไป

Background: Diabetes is a condition which is known to be associated with a variety of connective tissue abnormalities which contribute to impaired wound healing, leading to the chronic ulcer formation. *Centella asiatica Linn. and Garcinia mangostana Linn.* are widely used as Thai traditional medicine including wound treatment. However, the effects of both plant extracts in diabetic condition had not been experimented.

Objective: To study the effects of topical administration of *Centella asiatica Linn. and Garcinia mangostana Linn.* on diabetic wound.

Materials and Methods: Male Spraque-Dawley rats (64 rats) were divided into 2 groups: Normal control and diabetes induced groups, each group was divided into 4 groups. The wounds were and treated by placebo, *Centella asiatica Linn., Garcinia mangostana Linn.* and neomycin.

Results: It was shown that the wounds of the treated group epithelialized faster and the wound contraction rate was higher than the non-treated groups.

Conclusion: The present study results confirmed the folkloric utilization and suggested the beneficial effects of both plant extracts for treating diabetic wounds in human being. However, the further studies with purified constituents were required to understand the complete

mechanism of wound healing promotion induced by both *Centella asiatica Linn. and Garcinia mangostana Linn.*
Key words: wound healing, diabetes, *Centella asiatica Linn., Garcinia mangostana Linn.*

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Introduction

Wound healing is a complex process involving a highly regulated series of biological events. These include a set of co-ordinate interaction between cell in the dermis and epidermis. Diabetes is a condition which is known to be associated with a variety of connective tissue abnormalities. It contributes to impair wound healing, leading in many instances to chronic ulcer formation. Diabetic ulcers of the lower limbs and feet, in particular, are associated with high morbidity and often lead to the amputation[1]. Peripheral neuropathy and peripheral vascular disease are thought to be underlying factors in the diabetic wound formation[2], but dermal atrophy is likely to be a contributing factor in many cases[3]. Reduced fibroblast growth, increased expression of matrix-degrading matrix metalloproteinases (MMPs), and decreased matrix synthesis are the consequences of chronic vascular disease in diabetic skin as well as in other situations where peripheral vascular disease is present[4]. Atrophic skin is less resistant than healthy skin to wound formation. In addition, scanty extracellular matrix production during the proliferative phase of wound repair undoubtedly contributes to poor healing[5].

Centella asiatica Linn. has been shown to promote fibroblast proliferation and collagen synthesis[6] and to have antiulcer activity[7]. Shukla had also reported wound healing activity of the plant[8]. In primary screening an ethanolic extract of the plant showed significant wound healing activity. Repeated chromatography of the saponin mixture led to the isolation of two pure saponins identified as asiaticoside and madecassoside. Boiteau and Batsimamanga had reported the use of asiaticoside in healing experimental or refractory wounds[9], but the effect of this substance on wound healing process was not clearly understood.

Garcinia mangostana Linn, the fruit hulls have been in use in Thai folk medicine for the treatment of skin infections[10], wounds[11] and diarrhea[12]. Phytochemical studies showed that these plant species were rich in a variety of prenylated xantones[13] and the constituents had demonstrated a number of bioactivities[14]. Based on the actions for wound healing promotion activity of both Centella *asiatica Linn.* and *Garcinia* ***mangostana Linn.*** according to normal folklore, the evaluation of wound healing activity of both substances had been carried out.

Materials and Methods

Male Spraque-Dawley rats, weight 180-200 g were acclimated for 1 week upon arrival in the laboratory. Diabetes was induced by intraperitoneal injection of Streptozotocin (STZ ; 50 mg/kgBW in 0.2 ml of 10 mmol/l citrate buffer, pH 4.5). The rats were assessed blood glucose at 48 h after injection. They were regarded as diabetic when the blood glucose concentrations were > 250 mg/dL (measured by Glucometer). All rats were divided into 8 groups (8 rats/group). Group I (normal control) received placebo, Group II (normal control+*Centella asiatica Linn.* extract), Group III (normal control+*Garcinia mangostana Linn.* extract) and Group IV (normal control+neomycin) received neomycin, which is a standard drug for treatment of the diabetic wound. Group V, VI, VII and VIII are diabetic rats which received placebo, *Centella asiatica Linn.* extract, *Garcinia mangostana Linn.* extract and neomycin respectively.

Excision wounds were made on the 3rd day after induction of diabetes. They were cut on the left side of dorsal flank skin in the experimental rats. Animals were anesthetized with 40 mg/kg BW intraperitoneally of pentothal sodium and the left side of dorsal flank skin of each rat was shaved. Excision wounds were made by cutting out a 15 mm x 15 mm (225 mm^2) piece of skin from the shaven area. The wounds were full-thickness type, extending down to the subcutaneous tissue. All animal experiments were

carried out according to the guidelines and were approved by the local ethics animal review board.

Two types of Thai herbal plants that are commonly consumed in daily life were selected and collected between February and May 2008 from specific local sources in KhonKaen Province, Thailand. Each herbal plant was extracted with 95% ethanol as follows. For ethanol extraction, the fresh leaves of *Centella asiatica Linn.* 100 g and fruit hull of *Garcinia mangostana Linn.* 100 g were cleaned, chopped and macerated overnight in 100 ml 95% ethanol, respectively. The fluid so obtained were boiled in the oven until the volume of both solutions were approximately 10 ml, then cool down at the room temperature and mixed with approximated 90 ml glycerine gel.

Statistical analysis

The data are expressed as mean±SEM using analysis of variance followed by Student's *t*-test. Significance is calculated by comparing percentage of wound contraction in normal control groups and in diabetic groups. The value of $P < 0.05$ were considered significant. The percentage of wound contraction was calculated as a percentage of the corresponding day 0 (original) wound area (225 mm^2).

Results

The progress of wound healing induced in normal control groups treated by placebo (Group-I), *Centella asiatica Linn.* (Group-II), *Garcinia mangostana Linn.* (Group-III) and neomycin (Group-IV) are shown in Table 1. Time for wound closure as well as for falling of scab by placebo, gel and neomycin were comparable and all were shown the effective healing percentage after 4th days in gel-treated groups and 2nd, 8th days in neomycin treated group. However, the percentage of wound healing was compared between Group-I versus Group-II, Group-I versus Group-III and Group-I versus Group-IV and was found significantly different. Significant difference in the percentage of healing was observed from 2nd to 8th days of application (Table 1) and maximum difference was recorded after 4th, 6th and 8th days in gel-treated at 48.02%, 67.71%, 78.19% (Group-II) and 53.29%, 70.96%, 80.57% (Group-III) and 2nd, 8th days in neomycin treated at 6.10%, 41.38%, respectively.

Table 1 The percentage of wound contraction in normal control groups treated by placebo (Group-I), *Centella asiatica Linn.* (Group-II) *Garcinia mangostana Linn.* (Group-III) and neomycin (Group-IV).

Post wounding days	% of wound contraction			
	Group I	Group II	Group III	Group IV
2	18.73	22.26	26.44	6.10 #
4	33.49	48.02 †	53.29 *	20.49
6	50.00	67.71 †	70.96 *	35.77
8	64.76	78.19 †	80.57 *	41.38 #

Significance is calculated by comparing the percentage of wound contraction in Group-I vs. Group-II (?), Group-I vs. Group-III (*) and Group-I vs. Group-III (#) (†, * and # $p < 0.05$)

The percentage of wound contraction In diabetic groups treated by placebo (Group-V), *Centella asiatica Linn.* (Group-VI), *Garcinia mangostana Linn.* (Group-VII) and neomycin (Group-VIII) are shown in Table 2. Time for wound closure as well as for falling of scab by placebo, gel and neomycin were comparable and all were shown the effective healing percentage after 4th days in *Centella asiatica Linn.*-treated groups and after 2nd days in *Garcinia mangostana Linn.*-treated group. However, the percentage of wound healing was compared between Group-V versus Group-VI, Group-V versus Group-VII and Group-V versus Group-VIII, significance was found in Group-V versus Group-VI and Group-V versus Group-VII. Significant difference in the percentage of healing was observed from 2nd to 8th days of application (Table 2) and

Table 2 The percentage of wound contraction in diabetic groups treated by placebo (Group-V), *Centella asiatica Linn.* (Group-VI), *Garcinia mangostana Linn.* (Group-VII) and neomycin (Group-VIII).

Post wounding days	% of wound contraction			
	Group V	Group VI	Group VII	Group VIII
2	11.25	20.28	30.24 †	12.02
4	25.75	46.44 *	58.62 †	27.91
6	38.83	65.23 *	76.10 †	41.16
8	53.75	76.30 *	84.55 †	45.43

Significance is calculated by comparing the percentage of wound contraction in Group-V vs. Group-VI (*) and Group-V vs. Group-VII (†) (* and † $p < 0.05$)

maximum difference was recorded after 4th, 6th and 8th days in *Centella asiatica Linn.*-treated at 46.44%, 65.23%, 76.30% and after 2nd, 4th, 6th and 8th days in *Garcinia mangostana Linn.*-treated at 30.24%, 58.62%, 76.10%, 84.55%, respectively.

The present study also evaluated the effects of various interventions on histological features of the skin. The results showed that the size of the wounds in diabetes mellitus condition were bigger than those of normal condition at the same time of window treatment. Moreover, it also produced irritation, consisting of redness, dryness, and flaking with thinner epidermis and more pycnosis in the interstitial cells population. In the group of diabetic rats which received *Centella asiatica Linn.* extract or *Garcinia mangostana Linn.* extract were shown the increase of collagen that was an index of improvement of wound healing better than diabetic rats which received placebo or neomycin as shown in Figure 2.

Discussion

Healing is a physiological process and does not normally require much help but still wounds cause discomfort and are prone to infection and other complications. Wound healing has been reported to involve different phases such as epithelialisation, contraction, granulation, collagenation. Recent data also demonstrated that any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis[15]. Previous study showed that flavonoids could inhibit lipid peroxidaton, preventing or slowing the onset of cell necrosis, improved vascularity and promote the wound healing process mainly due to their astringent and antimicrobial property, which seemed to be responsible for wound contraction and increased rate of epithelialisation[16]. *Centella asiatica linn.*, has been studied for its wound healing activity in normal wound. The herbal was based on its traditional medicinal use and reported pharmacological activities such as promotion of fibroblast proliferation[17]. and stimulation of collagen synthesis[6]. In the present study, topical application of *Centella asiatica linn.* and *Garcinia mangostana Linn.* in diabetic wound significantly enhanced the rate of wound healing as assessed by increase in collagen synthesis and rate of wound contraction. Histological findings also showed enhanced proliferation of collagen. Angiogenesis plays an important role in wound healing and newly formed blood vessels comprise 60% of the repair tissue. Neovascularization helps hypoxic wounds to attain the normoxic conditions[18]. Based on these lines of evidence, the wound healing promotion activity of both herbal plant extracts observed in this study should be attributed to the function of either the individual flavonoid or the additive effects of the phytoconstituents existing in the herbal plant extract. However, the precise underlying mechanism and the possible active ingredient responsible for this effect are still required further investigation.

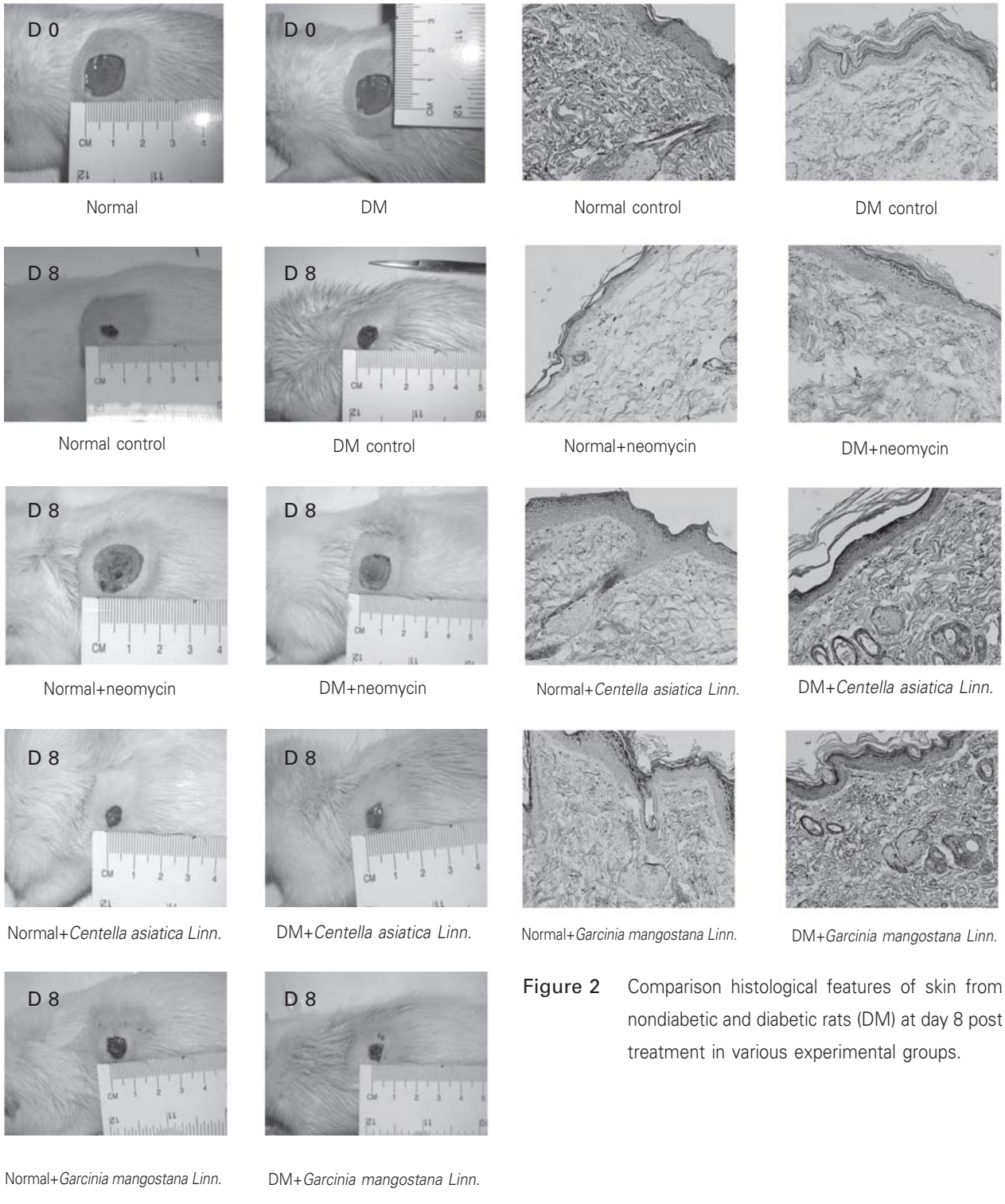

Figure 1 Photographical representation of contraction wounds on different treatment at day 8 (D 8) of nondiabetic and diabetic rats (DM) compared with day 0 (D 0).

Figure 2 Comparison histological features of skin from nondiabetic and diabetic rats (DM) at day 8 post treatment in various experimental groups.

Conclusion

This study demonstrates that both *Centella asiatica Linn.* and *Garcinia mangostana Linn.* could facilitate wound healing under diabetic condition in animal. Therefore, they may be suggested for treating diabetic wound in human being. However, the further studies with purified constituents were required to understand the complete mechanism of wound healing promotion induced by both *Centella asiatica Linn.* and *Garcinia mangostana Linn.*

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