Wound Healing Activity of Lawsoniainermis Linn in Rat Model

Sitthichoke Taweepraditpol MD*,
Vitusinee U-dee MD*, Sriprasit Boonvisut MD*,
Apirag Chuangsuwanich MD*, Kanaporn Pradniwat MD**

** Division of Plastic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Background: *Wound healing is the repairing process following the injury to the skin and soft tissues. Many kinds of wound dressings and agents are used for promoting the wound healing process.*
Objective: *To evaluate the effect of Lawsoniainermis Linn. A herbal extraction, in wound healing process.*
Material and Method: *In the present study Twenty Sprague Dawley rats were equally random into 4 groups. Each group were created similar excisional wound model and received 4 different kinds of wound dressings including Lawsoniainermis Linn., Silver Zinc sulfadiazine and hydrogel. We compared wound size reduction and rate of epithelalization and also studied histological change of the wound.*
Results: *The wound size reduction and rate of epithelialiazation were significantly better in Lawsoniainermis Linn comparing to the other groups. Also, Histological study showed a significant increase in collagen and fibroblasts and decrease inflammatory cells.*
Conclusion: *Lawsoniainermis Linn can promote the process of wound healing in animal model and should be further studied in the future.*

Keywords: *Lawsoniainermis Linn, Wound healing*

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After skin and soft tissues injury, wound healing process occurs in three phases: inflammatory, proliferation, and remodeling phase[1]. In proliferation phase, angiogenesis, collagen deposition, epithelization and wound contraction occur. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new provisional extracellular matrix by excreting collagen and fibronectin. In epithelialization, epithelial cells proliferate across the wound surface and wound contraction occurs as the result of myfibroblast contraction.

Until now, the research in wound healing has not been yielded a safe, economic, and efficacious prohealing agent that could obviate the long hospitalization of patients suffering from wound. So, screening of natural resources as one of the strategies for identification of such agents would seem highly beneficial.

Lawsoniainermis Linn belongs to family Lythraceae, and commonly called Henna, Mehndi, Mendi. Product of this plant is generally used for a traditional cosmetic agent. People not only use as a cosmetic agent to stain the hair, skin and nails but also apply to the body on lesions in the treatment of seborrheic dermatitis and fungal infections. In this study, we would like to evaluate the effects of Lawsoniainermis Linn preparation, herbal extraction, in wound healing.

Correspondence to:
Taweepraditpol S, Division of Plastic surgery Department of Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Phone: +66-2-4198002, Fax:+66-2-4199641
E-mail: ntdclub13@gmail.com

Material and Method

This prospective randomized controlled animals studied was approved by Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC). They were maintained according to the "Guide for the care and Use of Laboratory Animals" established by National Laboratory Animal Center of Mahidol University, Thailand

Animals

Twenty eight-week-old healthy male Sprague

Dawley rats from the Faculty of Veterinary Science, Mahidol University, and weight between 250 to 280 grams were enrolled. They were equally random allocated into 4 groups.

Excisional wound model (Fig. 1)

1) Prior to infliction of the experimental wounds under sterile conditions as described by Morton and Malone[2], each rat was anesthetized using intraperitoneal injection of 40 to 50 mg/kg combined Tiletamine-Zolazepam and buprenorphine 0.01 to 0.05 mg/kg, subcutaneously.

2) Prophylactic antibiotics treatment using enrofloxacin 5 to 10 mg/kg, subcutaneously.

3) The skin over the dorsal area was shaved completely and application field was marked with a pen. A full thickness of the excision wound (area 400 mm^2 and 2 mm depth) was created (Fig. 2).

4) Then the wound was cleaned with normal saline and applied wound dressing according to the group and assessed until completely healed.

Group C (control): dressing with normal saline soaked sterile gauze.

Group S: applied with Silver Zinc sulfadiazine cream before covered with sterile gauze once daily.

Group H: applied with hydrogel (Intrasite gel, Smith & Nephew Ltd) before covered with sterile gauze once daily.

Group L: sprayed with Lawsoniainermis Linn 8 times before covered with sterile gauze twice a day.

Lawsoniainermis Linn preparation

Lawsoniainermis Linn spray was prepared by Government Pharmaceutical Organization (GPO). Fresh henna leaves (Lawsoniainermis Linn, Fig. 2) were collected by National Swine Research and Training Center, Suwanvajokkasisit Animal Research and Development Institute, Thailand. After cleaning the leaves with water and air dried then blended the leaves with 16% ethanol. After that, extract of Lawsoniainermis Linn was filtered. The clear filtrate was used for further preparation. Excipients,,according to GPO formulation, consisting of sugar and salt were added in the extract of Lawsoniainermis Linn in ratio 1: 9 to form a Lawsoniainermis Linn preperation for this study.

Wound size measurement

Each wound area was measured on post-excision day 1, 5, 10 and 15 using a Visitrak (Trademarks of Smith & Nephew); a portable digital device, and observed until completed epithelialization.

Histological evaluation

Each wound edge was biopsied at on post-excision day 5, 10 and 20. Then it was fixed in 10% buffered formalin. Each specimen was embedded in a paraffin block and a thin section (3 μm) was prepared and stained with hematoxylin-eosin method.

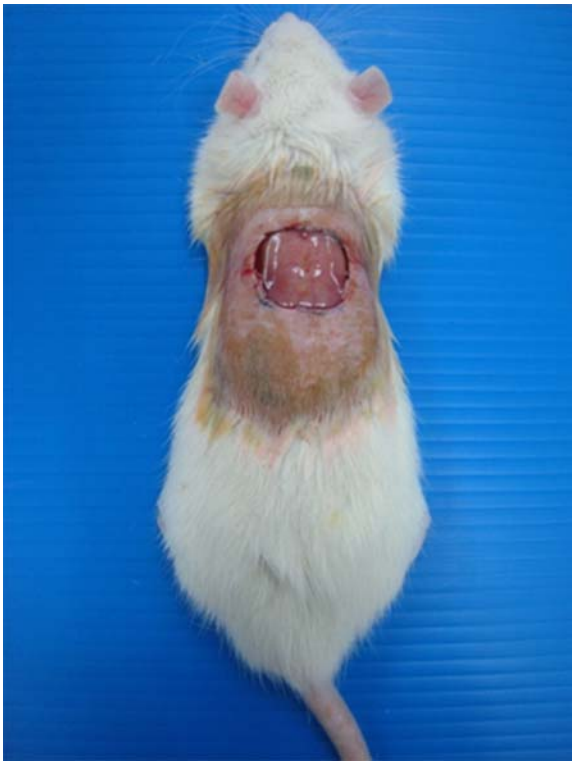

Fig. 1 Excisional wound model.

Fig. 2 Fresh henna leaves (Lawsoniainermis Linn).

Statistical analysis

The mean of wound area and the day that the wound had completed epithelialization between each group are compared with Kruskal-Wallis test. In case of statistical significant difference among 4 groups, Mann-Whitney U test is then performed for pairwise comparisons. Data are analyzed using SPSS version 17 and *p*-value of <0.05 is set for all analysis.

Results

Wound size measurement

Lawsoniainermis Linn Preparation in this study showed faster wound size reduction than other groups significantly. The mean of wound size, at the 5th day, the 10th day and the 15th day in each group were shown in Table 1. Whereas Table 2 shows relative wound size reduction in each rat over time separately for each group. Group L has showed a significant relative reduction in wound area (88%, 96%) when compared with group C (77%, 87%), group S (82%, 92%) and group H (72%, 89%).

Comparison of wound size reduction among four groups at the 15th day showed that the relative reduction in wound size in group L was significantly greater than group C, group S and group H (Table 3).

Rate of epithelization

Regarding to the rate of epithelialization animal in Group L have showed faster rate of epithelialization (mean ± SD, days: 12.0±0.70 respectively) compared to group Cand group H, and epithelialization rate of Group Lhave showed equivalent rate of epithelialization compared to Group S (mean ± SD, days: 12.20±0.44) (Table 4).

Histological evaluation

Histological studies of the tissue that obtained from wound edge at day 20th has showed that group L had a significant increase in collagen and fibroblast and decrease inflammatory cell more than the other groups (Fig. 3).

Discussion

Lawsoniainermis Linn in family Lythraceae, and in common names: Henna, Mehndi, Mendi. L. inermis is a traditional cosmetic agent and is used worldwide; it is not only as a cosmetic agent to stain the hair[3], skin and nails but also applied to the body on lesions in the treatment of seborrheic dermatitis or fungal infections. The leaves of Lawsoniainermis Linn contain lawsone (2-hydroxy-1, 4-naphthoquinone),

Table 1. Show wound area (mm^2): Mean ± SD in each group

	Wound area (mm^2): mean ± SD			
Group	Day1st	Day5th	Day10th	Day15th
C	388±8.37	298±37.01	86±11.40	50±15.81
S	384±11.40	234±43.94	66±23.02	30±7.07
H	384±11.40	296±37.82	104±41.59	40±18.71
L	386±5.48	258±33.47	46±11.40	12±4.47
p-value	0.932	0.171	0.033	0.012

Table 2. Percentage reduction in wound area in each group

	% Relative reduction in wound area: mean ± SD		
Group	(D1-D5)/D1	(D1-D10)/D1	(D1-D15)/D1
C	23.19±9.38	77.83±2.95	87.12±4.09
S	39.27±9.59	82.90±5.66	92.20±17.23
H	22.91±9.60	72.84±11.07	89.50±5.02
L	33.19±8.19	88.05±3.08	96.89±1.13
p-value	0.140	0.035	0.021

Table 3. Multiple comparisons of % reduction in wound area at day 15th among 5 groups

	Exact *p*-value			
Group	C	S	H	L
C		0.056	0.460	0.008*
S			0.397	0.008*
H				0.016*
L				

Table 4. Comparison of rate of epithelialization among 5 groups

		Epithelialization time (days)		
Group	n	Mean±SD	Min,Max	*p*-value
C	5	15.20±0.44	15,16	<0.01
S	5	12.20±0.44	12,13	
H	5	15.20±0.44	15,16	
L	5	12.00±0.70	11,13	

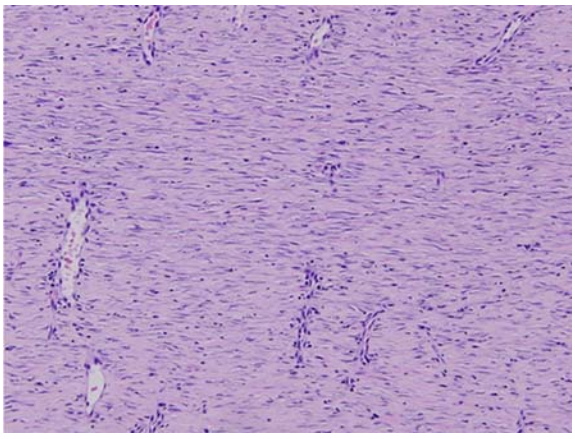

Fig. 3 Histopathology at day 20th from wound edge of group L.

gallic acid, glucose, mannitol, fat, resin and mucilage[4].

In 2005, HS Muhammad and S Muhammad proved that L inermis has a valuable role in the management of infected burn wounds[4]. In 2007, B. Shivananda Nayak et al proved that ethanol extract of L. inermis Linn can promote wound healing activity, enhance wound contraction, tensile strength, and increase hydroxyproline content[5]. L. inermis also has a wide spectrum of antimicrobial activity including antibacterial, antiviral, antimycotic and antiparasitic activity[6]. Thai Traditional medicine uses leaves of the L. inermis for many treatments including abrasion wound, burn wound, infected wound and paronychia.

Our study has shown that Lawsoniainermis Linn preparation can reduce in wound size more than other group significantly. Also rate of epithelialization is faster than control group and hydrogel group but had equivalent rate of epithelialization to silver sulfadiazine group.

Histological results also support the evidence of improvement in wound healing, tissue that obtained from Lawsoniainermis Linn preparation in rat had a significant increase in collagen and fibroblast and decreased inflammatory cell more than others group.

Conclusion

Lawsoniainermis Linn. might be an another option to accelerate wound healing process. However, further studies in active pharmacological component and clinical trial should be needed in the future.

What is already known on this topic?

There are several experimental studies about Lawsoniainermis Linn. Extract in wound healing and antimicrobial activity.

What this study adds?

In this study, the effect of Lawsoniainermis Linn. Extract in wound healing was compared to other

standard wound dressing. This herbal extract might be studied further in clinical trials. It can be considered another optional wound dressing in the future.

Potential conflicts of interest

None.

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การศึกษาเปรียบเทียบการหายของบาดแผลที่ให้การรักษาด้วยสารสกัดจากใบเทียนกิ่ง (Lawsoniainermis Linn GPO preparation) ในสัตว์ทดลอง

สิทธิโชค ทวีประดิษฐ์ผล, วิทูสินี อยู่ดี, ศรีประสิทธ์ บุญวิสุทธิ์, อภิรักษ์ ช่วงสุวนิช, คณาพร ปราชญ์นิวัฒน์

ภูมิหลัง: *กระบวนการหายของบาดแผลเป็นกระบวนการซ่อมแซมของร่างกายภายหลังเกิดการบาดเจ็บที่ผิวหนังและเนื้อเยื่อ ปัจจุบันมีอุปกรณ์ทำแผลหลายชนิดมากที่ใช้สำหรับการส่งเสริมกระบวนการหายของแผลให้ดีขึ้น แต่ในปัจจุบันยังไม่มีอุปกรณ์ทำแผลชนิดใดมีความปลอดภัยและมีประสิทธิภาพที่ดีที่สุด*

วัตถุประสงค์: *เพื่อประเมินผลของสารสกัดจากใบเทียนกิ่ง (Lawsoniainermis Linn) ที่มีต่อกระบวนการหายของบาดแผล เมื่อเทียบกับซิลเวอร์ซัลฟาไดอะซีน (silver sulfadiazine) และไฮโดรเจล (Hydrogel) ในหนูทดลองโดยดูจากระยะเวลาการหายของบาดแผลและลักษณะ ทางพยาธิวิทยา*

วัสดุและวิธีการ: *ในการทดลองนี้ศึกษาผลต่อแผล excisional ของสารสกัดจากใบเทียนกิ่ง (Lawsoniainermis Linn) เปรียบเทียบกับ Silver sulfadiazine และ Hydrogel ในหนูทดลองโดยศึกษาจากการลดขนาดระยะเวลาการหายและลักษณะทางพยาธิวิทยาของแผล*

ผลการศึกษา: *พบว่ามีการลดขนาดและหายของแผลที่ดีกว่าอย่างมีนัยสำคัญในกลุ่มที่ได้รับการรักษาด้วยสารสกัดจากใบเทียนกิ่ง (Lawsoniainermis Linn) เปรียบเทียบกับกลุ่มอื่น ๆ รวมทั้งมีการเพิ่มการสร้าง เนื้อเยื่อคอลลาเจนมากขึ้นอย่างมีนัยสำคัญด้วย*

สรุป: *สารสกัดจากใบเทียนกิ่ง (Lawsoniainermis Linn) สามารถส่งเสริมกระบวนการของการสมานแผลในสัตว์ทดลองและควรมีการศึกษาเพิ่มต่อไปในอนาคต*
