ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

Wound healing and antimicrobial activity of two classical formulations of *Laghupanchamula* in rats

Shivani Ghildiyal[1], Manish K. Gautam[2], Vinod K. Joshi[1], Raj K. Goel[1]

Departments of [1]Dravyaguna and [2]Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

ABSTRACT

Background: Wounds affect a large number of patients and seriously reduce the quality of life. The wound as a medical problem was first discussed by *Maharshi Agnivesha* in *Agnivesha Samhita* (later known as *Charaka Samhita*) as *Vrana*. *Laghupanchamula* denotes a combination of the roots of five herbs. However, in *Ayurvedic* classics, besides four common herbs viz. *Kantakari*, *Brihati*, *Shalaparni* and *Prinshniparni*, the fifth one is either *Gokshura* (LPG) or *Eranda* (LPE), and both formulations have been documented to have wound healing (*Vrana*) activity. **Objective:** The present study was undertaken to determine the *in vivo* wound healing activity and *in vitro* antimicrobial activity of 50% ethanolic extract of *Laghupanchamula* containing *Gokshura* (LPGE) and *Laghupanchamula* containing *Eranda* (LPEE) in rats with acute toxicity in mice. **Materials and Methods:** LPGE and LPEE (1000 mg/kg) was administered orally, once daily for 10 days (incision wound model) or for 24 days (excision wound model) in rats. LPGE and LPEE was studied for its *in vitro* antimicrobial and *in vivo* wound breaking strength (WBS) (incision model) and rate of contraction, period of epithelization and histology of skin (excision model). **Results:** LPGE and LPEE showed antimicrobial activity against skin pathogens, enhanced WBS, rate of contraction, skin collagen tissue formation and early epithelization period with low scar area indicating enhanced healing with histological evidence of more collagen formation in skin tissues. LPGE and LPEE also showed anti-bacterial activity and seemed to be safe. **Conclusion:** Use of both formulations in *Laghupanchamula* for their wound healing and anti-microbial activities is thus authenticated.

Key words: Antimicrobial, excision wound, incision wound, *Laghupanchamula*, minimum inhibitory concentration

INTRODUCTION

Wounds are major concerns for the patient and clinician alike. Healing of wounds is one of the important areas of clinical medicines explained in many *Ayurvedic* texts under the heading *Vranaropaka*. The wound as a medical problem was first discussed by *Maharshi Agnivesha* in *Agnivesha Samhita* (later known *as Charaka Samhita*) as *Vrana*. *Maharshi Sushruta* in *Sushruta Samhita* elaborated more on wound and its healing. According to the *Ayurveda*, *Vrana* is the discontinuation of the lining membrane, which, after healing, leaves a scar for life closely resembling the modern definition. Similarly, inflammation is considered to be an early phase in the pathogenesis of wounds termed *Vranashotha*. Different types of wounds as mentioned in *Ayurveda,* may be either endogenous in origin due to a defect in human functional units, such as *Vata*, *Pitta* and *Kapha*, or exogenous due to trauma, such as *Chhinna* (cut wound), *Bhinna* (perforated wound), *Viddha* (punctured wound), *Kshata* (lacerated wound), *Picchita* (contusion) and *Ghrista* (abrasion wound).[1]

Address for correspondence:
Prof. Raj K. Goel,
Department of Pharmacology, Faculty of Modern Medicine,
Institute of Medical Sciences, Banaras Hindu University,
Varanasi - 221 005, Uttar Pradesh, India.
E-mail: rkgoelbhu50@gmail.com

Received: 26-Jul-2014
Revised: 01-Sep-2014
Accepted: 15-Nov-2014

Access this article online

Quick Response Code:

Website: www.jaim.in

DOI: 10.4103/0975-9476.157952

How to cite this article: Ghildiyal S, Gautam MK, Joshi VK, Goel RK. Wound healing and antimicrobial activity of two classical formulations of *Laghupanchamula* in rats. J Ayurveda Integr Med 2015;6:241-7.

Laghupanchmula is a group of five small plants' (herbs or undershrubs) roots. Two different classical combinations of *Laghupanchmula* have been advocated each containing roots of four common plants, *Kantakari* (*Solanum surratense* Burm f.), *Brihati* (*Solanum indicum* Linn.), *Shalaparni* (*Desmodium gangeticum* DC.) and *Prinshniparni* (*Uraria picta* Desv.). The fifth plant is eithe *Gokshura* (*Tribulus terrestris* Linn.) as advocated in *Charaka Samhita*[2], "*Chakradatta*,"[3] *Shargadhara Samhita*, *Vangasena Samhita*, *Yogaratnakara* and *Bhaisajyasatnavali* (LPG, the first compound formulation) or roots of *Eranda* (*Ricinus communis* Linn.) as advocated in *Sushruta Samhita*,[4] *Kashyapa Samhita*[5] and *Siddhasara Samhita*[6] (LPE, the second compound formulation). The plants included under *Laghupanchamula* have been explored individually for various pharmacological properties. *Solanum surratense* Burm f. juice mixed with whey, chiretta and ginger has been reported to be useful in fever. The juice of leaves, mixed with black pepper, has been prescribed in rheumatism.[7] *Solanum indicum* Linn. root has been reported to have hepatoprotective, anti-inflammatory and wound healing activities.[8,9] *Desmodium gangeticum* roots have been documented for therapeutic value in treating typhoid, piles, inflammation, asthma, bronchitis and dysentery.[10] *Uraria picta* Desv. root has been reported for its analgesic, anti-inflammatory, antioxidant, aphrodisiacs and fracture-healing properties.[11] *Tribulus terrestris* Linn. has been reported in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic and urinary anti-infective.[12] *Ricinus communis* Linn seeds, seed oil, leaves and root have been reported for the treatment of inflammation and liver disorders.[13] However, wound healing activity of the above two formulations of *Laghupanchmula*, i.e. LPG and LPE, has not been explored earlier. Therefore, a comparative evaluation of 50% ethanolic extract of LPG (LPGE) and LPE (LPEE) for their wound healing (incision and excision wound models) activity in rats and *in vitro* antimicrobial activity on common skin bacteria was evaluated.

MATERIALS AND METHODS

Animals

Charles–Foster strain albino rats (150–200 g) and Swiss albino mice (20–30 g) of either sex (n = 6) were obtained from the Central Animal House of the Institute, Banaras Hindu University, Varanasi. They were kept in the departmental animal house at 24 ± 2°C and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with a standard rodent pellet diet. Principles of laboratory animal care (NIH publication no. 82–23, revised 1985) guidelines were followed. Ethical permission for the investigation of animals used in experiments was taken from the Animal Ethics Committee of the Institute (Notification No. Dean/2012-13/CAEC/214 dated 29.09.2012).

Collection of plant materials

Roots of *Kantakari, Brihati, Shalaparni, Prinshniparni, Eranda* or *Gokshura* whole plant were collected from the Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur in the months of November to December 2009 and a sample specimen of each preserved in the Department of Dravyaguna, IMS, BHU, Varanasi.

Preparation of plant extracts

The collected roots of the above-mentioned plants were shade dried and reduced to a coarse powder using a mechanical grinder and stored in air-dried containers. Two hundred grams each of the dried powder of the roots of *Kantakari*, *Brihati*, *Shalaparni* and *Prinshniparni*, plus either *Gokshura* i.e. LPG (first formulation) or *Eranda* i.e. LPE (second formulation), were taken. Extracts of the above-mentioned two formulations of *Laghupanchamula* (1 kg each) named as LPGE and LPEE were prepared separately in a soxhlet apparatus using 50% ethanol following the standard procedures. The percentage yields of LPGE and LPEE were 10.92% and 10.78%, respectively.

Drugs and chemicals

Vitamin E was obtained from Merck Ltd., Mumbai, India and all the other chemicals and reagents used were of analytical grade.

Dose selection and treatment protocol

A preliminary dose–response effect using LPEE and LPGE was first undertaken to study the wound breaking strength (WBS) in incision wound models in rats. Graded doses of LPEE and LPGE 500, 1000 and 1500 mg/kg were administered once daily orally for 10 days in rats following induction of incision wound. The above doses were selected on the basis of our previous reported anti-inflammatory studies with LPEE and LPGE.[14] The sutures were removed on the 7th day of the experiment and

Table 1: Effect of LPGE, LPEE and Vitamin E on wound breaking strength

Oral treatment (mg/kg, once daily × 10 days)	Wound breaking strength (g)
Control 0.5% CMC	400.0±11.5 (100.0±2.89)
LPGE 500	422.17±18.96[a] (105.5±4.74)
LPGE 1000	563.3±16.3[c] (140.8±4.06)
LPGE 1500	569.8±17.4[c] (142.5±4.35)
LPEE 500	440.67±17.08[b] (110.17±4.27)
LPEE 1000	528.3±18.5[c] (132.1±4.69)
LPEE 1500	538.8±18.57[c] (134.7±4.64)
VTE 200	550.0±16.3[c] (137.5±4.08)

Results are mean±SEM (% WBS) of six rats in each group. [a]P<0.05, [b]P<0.01 and [c]P<0.001 compared with the control group (statistical analysis was performed by one-way analysis of variance followed by the Dunnett's test for multiple comparisons). LPGE=*Laghupanchamula* containing *Gokshura*, LPEE=*Laghupanchamula* containing *Eranda*, CMC=carboxy methyl cellulose, VTE=Vitamin E, WBS= wound breaking strength

WBS was measured on the 10th post-wounding day. The result of the dose–response study in the incision wound model indicated that 1000 mg/kg of LPEE and LPGE had an optimal effect [Table 1]. Therefore, the dose of 1000 mg/kg of LPEE and LPGE was chosen for their further study on healing effects on the excision wound model.

LPEE, LPGE (1000 mg/kg) and the standard drug Vitamin E (VTE; 200 mg/kg) suspended in 0.5% carboxy methyl cellulose (CMC) in distilled water were given orally once daily from Day 1, 4 h after the induction of the excision wound. The animals received the extract/VTE orally with the help of an oro-gastric tube in the volume of 1 mL/100 g body weight. For excision wound study, drugs were given for 22 days or earlier till complete healing day, while control rats received 0.5% CMC during the study period.

Wound healing studies

Incision wound model

Two parallel 6 cm paravertebral incisions were made through the full-thickness of the skin, 1 cm lateral to the midline of the vertebral column after giving anesthesia (Ketamine hydrochloride, 50 mg/kg). Wounds were closed with interrupted sutures, 1 cm apart, with a surgical suture. The sutures were removed on the 7th post-wounding day. WBS was measured on the 10th post-wounding day in anesthetized rats. A line was drawn on either side of the incision line 3 mm away from the wound. Two Allis forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Standard weights were put slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the weight was stopped and noted.[15]

Excision wound model

Rats were anesthetized with ketamine, an area of about ≈500 mm^2 was marked on the back of the rat by a standard ring and then the full thickness of the marked skin was cut carefully. Wounds were traced on a 1 mm^2 graph paper on the day of wounding and subsequently at a gap period of 4 days till the 12th day, then on the alternate days until healing was complete. Changes in wound area were measured regularly and the rate of wound contraction was calculated as given in the formula below. Significance in wound healing of the test groups are derived by comparing the healed wound area on respective days with the healed wound area of the control group. The period of epithelization, i.e. day of fall of eschar, and the scar area were also noted down.[16]

$$\% \text{ wound contraction} = \text{healed area}/\text{total wound area} \times 100$$

Where, healed area = original wound area - present wound area.

Antimicrobial susceptibility and minimum inhibitory concentration

In vitro antibacterial susceptibility test of LPGE and LPEE was performed using serial concentrations of 50, 100, 150 and 200 mg/mL following the approved standards of the National Committee for Clinical Laboratory Standards against common skin bacteria *Staphylococcus aureus* (ATCC 25323), *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* obtained from the American Type Culture Collection (ATCC) and clinical strain preserved at the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India following the disk diffusion method,[17] while the MIC was performed by the microdilution method.[18]

Limit test study

Adult Swiss albino mice of either sex, weighing between 20 and 25 g, fasted overnight, were used for the toxicity study. A suspension of LPGE and LPEE was administered orally at 5 g/kg stat dose (five-times of the optimal effective dose) to mice. Subsequent to the administration of the extracts, animals were observed closely for the first 3 h, for any manifestation of toxicity, like increased motor activity, salivation, convulsion, coma and death. Subsequently, observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 2 weeks.[19,20]

Statistical analysis

Statistical comparison was performed using either unpaired *t*-test or one-way analysis of variance (ANOVA) for multiple comparisons versus the control group was carried out by Dunnett's test. All statistical analyses were performed using SPSS statistical version 16.0 software package (SPSS® Inc. Armonk, New York, USA).

RESULTS

Incision wound

Control rats (0.5% CMC) showed WBS as 400.0 ± 11.5 g on the 10th post-wound day. LPGE 500, 1000 and 1500 mg/kg treated rats showed WBS as 422.17 ± 18.96, 563.3 ± 16.3 and 569.8 ± 17.4 g, respectively, while LPEE 500, 1000 and 1500 mg/kg treated rats showed WBS as 440.67 ± 17.08, 528.3 ± 18.5 and 538.8 ± 18.57 g, respectively. Both

these extracts showed a significant increase in WBS when compared with the control group, and this was comparable with 200 mg/kg of VTE (WBS as 550.0 ± 16.3 g). After analyzing the results of the above three different doses of LPGE and LPEE on WBS parameter in incision wound model, an optimal effective dose of 1000 mg/kg both for LPGE and LPEE was selected for further studies in the excision wound model in rats [Table 1].

Excision wound

The rate of wound contraction in the control rats was 24.0%–63.9% from Day 4 to Day 12 and 78.3–98.0% from Day 14 to Day 20, while complete epithelization and healing was observed on Day 22. The average number of days that took for the shedding of eschar without leaving any residual raw wound in these rats was 11.2 days, and the mean of the scar area was 185.7 mm^2. The percent rate of wound contraction in rats treated with LPGE was from 32.1% on Day 4 to 80.2% on Day 12 and 90.2–100% from Day 14 to Day 20. The percent rate of wound contraction in rats treated with LPEE was from 33.5% on Day 4 to 75.6% on Day 12 and 85.0% to 99.5% from Day 14 to Day 20, while complete wound healing was observed on the 21st day. VTE treated rats showed an increase in wound contraction from 35.4% on Day 4 to 80.3% on Day 12 and 89.2–100% from Day 14 to Day 20. LPGE and LPEE showed a time-dependant healing effect on wound surface area in excision wound model in rats. The area under the curve (AUC) of the LPGE- and LPEE-treated groups versus the control group gave an indication of faster healing, which was comparable with that of the VTE-treated group. The mean epithelization period and scar area observed with LPGE and LPEE were 9.17 days and 88.8 mm^2 and 8.67 days and 114.7 mm^2, respectively, while the mean epithelization period and scar area observed with VTE were 8.17 days and 88.3 mm^2, respectively [Table 2, Figure 1].

Histology of excision biopsy of skin wound at day 10 showed healed skin structures with normal epithelization, restoration of adnexa and fibrosis within the dermis in LPGE, LPEE and VTE treated groups while the control group lags behind treated groups in formation of the amount of ground substance in the granulation tissue [Figure 2].

Antimicrobial susceptibility and MIC

The LPGE and LPEE extracts showed a positive susceptibility test against the common skin bacteria *Staphylococcus aureus* (ATCC 25323), *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, showing a zone of inhibition ≥10 mm at 200 mg/mL. LPEE had the lowest MIC of 1.57 mg/mL against *Staphylococcus aureus* (ATCC 25323) and 3.13 mg/mL against *Pseudomonas aeruginosa*, whereas the MIC of LPGE and LPEE against other organisms ranged from 6.25 to 25.0 mg/mL [Table 3].

Limit test study

LPGE and LPEE, even at five-times their optimal effective doses, i.e. 5 g/kg, when given in stat dose, orally, did not

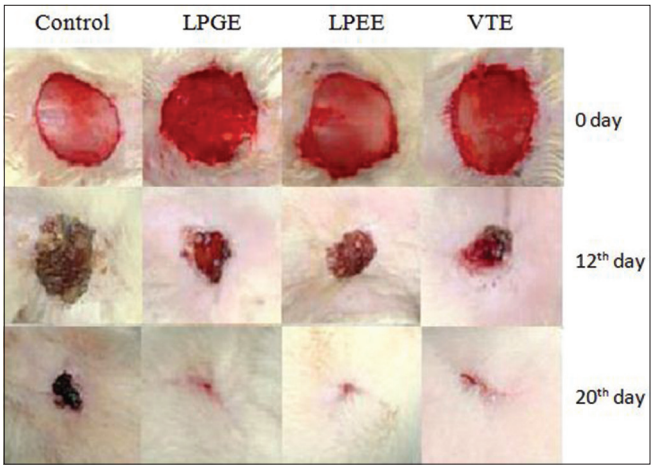

Figure 1: Photographic representation of contraction rate showing percent wound contraction area on different post-excision days of control, *Laghupanchamula* containing *Gokshura* (LPGE) and *Laghupanchamula* containing *Eranda* (LPEE) (1000 mg/kg) and Vitamin E (VTE) (200 mg/kg) treated rats

Table 2: Effect of LPGE, LPEE and Vitamin E on wound contraction, epithelization period and scar area

Oral treatment (mg/kg, once daily)	Wound area in mm^2/rat (% contraction)									Epithelization period (days)	Scar area (mm^2)
	0 day	4th day	8th day	12th day	14th day	16th day	18th day	20th day	22nd day		
Control 0.5% CMC	554.3±11.2 (0.0±3.26)	421.5±9.87 (24.0±1.78)	309.7±6.39 (44.1±1.15)	200.2±6.01 (63.9±1.08)	120.5±5.98 (78.3±1.07)	78.5±2.42 (85.9±0.43)	38.7±1.06 (93.0±0.19)	11.33±0.67 (98.0±0.12)	3.50±0.56 (99.4±0.10)	11.2±0.31 (100±2.77)	185.7±9.00 (100±0.05)
LPGE 1000	512.3±7.56^b (0.0±1.48)	348±5.98^c (32.1±1.16)	210.5±5.44^c (59.0±1.06)	101.5±3.68^c (80.2±0.71)	49.8±3.64^c (90.2±0.71)	17.0±1.63^c (96.7±0.32)	5.67±0.67^c (99.0±0.22)	1.50±0.50^c (100.0±0.09)	0.0±0.0^c (100.0±0.0)	9.17±0.31^c (81.9±2.77)	88.8±4.41^c (47.8±2.37)
LPEE 1000	535.0±5.12 (0.0±0.96)	355.8±6.42^c (33.5±1.2)	256.5±8.7^c (52.1±1.62)	130.3±10.5^c (75.6±1.96)	80.5±5.40^c (85.0±1.00)	27.0±2.43^c (95.0±0.45)	10.0±0.82^c (98.1±0.15)	2.83±0.54^c (99.5±0.10)	0.0±0.0^c (100.0±0.0)	8.67±0.33^c (77.4±2.95)	114.7±10.2^c (0.67±8.89)
VTE 200	522±5.42^a (0.0±1.03)	337.5±5.90^c (35.4±1.13)	179±7.31^c (65.8±1.40)	102.7±4.0^c (80.3±0.77)	56.3±5.07^c (89.2±0.97)	26±3.30^c (95.0±0.63)	9.33±1.11^c (98.2±0.21)	2.17±0.83^c (100.0±0.15)	0.0±0.0^c (100.0±0.0)	8.17±0.31^c (72.9±2.77)	88.3±5.67^c (47.5±3.05)

Values are mean±SEM of six rats in each group. Values in parenthesis indicate percent decrease from the Day 0 value of the respective group. ^aP<0.05, ^bP<0.01 and ^cP<0.001 compared with the respective day control group (statistical analysis was performed by one-way analysis of variance followed by the Dunnett's test for multiple comparisons). LPGE=*Laghupanchamula* containing *Gokshura*, LPEE=*Laghupanchamula* containing *Eranda*, CMC=carboxy methyl cellulose, VTE=Vitamin E

show any acute toxic manifestations like increased motor activity, compulsive behavior, salivation, convulsions, coma or death when observed up to a period of 2 weeks.

DISCUSSION

The plants included under *Laghupanchamula* have been explored individually for various pharmacological properties, but no such comparative study was performed earlier with the two formulations of *Laghupanchamula* that were advocated in the *Ayurvedic* compendia for various inflammatory ailments. Presently, a comparative study was performed with the two formulations of LP on their wound healing activity using incision and excision wound models in rats and also *in vitro* antimicrobial activity against some common skin microorganisms.

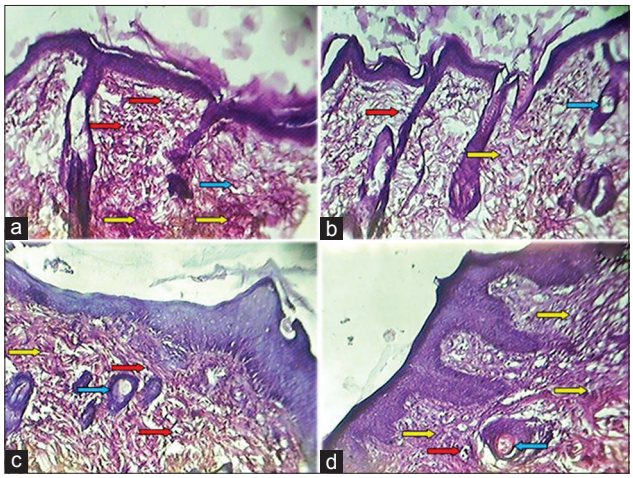

Figure 2: Histopathology of skin tissue at Day 10 stained with hematoxylin and eosin (x20). (a) Skin tissue of carboxy methyl cellulose-treated control rat showed extensive damage with ulcerated epidermis filled with only granulation tissue comprising of mononuclear inflammatory cells (red arrow), collagen tissue (yellow arrow) and blood vessels (blue arrow), (b) Vitamin E (200 mg/kg)-treated rats showed near-normal epidermis with mild to moderate mononuclear infiltrate in the underlying dermis and few blood vessels, (c) skin tissue of *Laghupanchamula* containing *Gokshura* (1000 mg/kg)-treated rat showed mild mononuclear inflammatory cells (red arrow), abundant collagen tissue (yellow arrow) and few blood vessels (blue arrow) and (d) *Laghupanchamula* containing *Eranda* (1000 mg/kg)-treated rat shows moderate amount of mononuclear infiltrate interspersed with thin-walled blood vessels

Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue: Inflammatory, proliferation and remodeling stages.[21] The inflammation stage begins immediately after injury, first with vaso-constriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibers that increases the tensile strength.[22] Thus, it is generally believed that alterations in the first phase, which is the inflammatory one, will impact the overall integrity of the healing wound.[23] The involvement of each phase has been reported to vary over a spectrum dependent largely on the type, location and milieu factors influencing the wound. The healing process thus depends, to a large extent, on the regulated biosynthesis and deposition of new collagens and their subsequent maturation.[15] In our present incision wound study, both the extracts showed increased WBS as compared with the control. In this model, the increase in tensile strength of the treated wounds may be due to the increase in collagen concentration and stabilization of the fibers.[24] It may be postulated that the collagen molecules synthesized at the wound site are laid down and become cross-linked to form fibers. Wound strength is thus acquired from both remodeling of collagen and the formation of stable intra- and inter-molecular cross-links. Because incision wounds treated with LPGE and LPEE showed greater breaking strength, it may increase remodeling of collagen and the formation of stable intra- and inter-molecular cross-links.[25]

Epithelization is the process where keratinocytes migrate from the lower skin layers and divide. Contraction is the process where the wound contracts, narrows or closes the wound. The movement of fibroblast s in the wound area facilitates matrix formation and collagen is laid down over and throughout the amorphous material. In the tissue repair process, inflammatory cells promote the

Table 3: Antibacterial activity and minimum inhibitory concentration of LPGE and LPEE

Name of organism	Antibacterial activity (zone of inhibition in mm)					MIC (mg/mL)
	Extract	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	
Staphylococcus aureus (ATCC 25323)	LPGE	9.3±0.57	8.1±0.94	10.6±1.24	11.5±1.63	12.5
	LPEE	7.9±1.63	8.5±1.24	10.2±0.47	11.1±0.21	1.57
Staphylococcus epidermidis	LPGE	7.6±0.62	8.8±0.30	9.4±0.86	10.5±0.20	25.0
	LPEE	8.1±0.32	9.4±0.30	10.1±0.42	10.9±0.36	12.5
Pseudomonas aeruginosa (ATCC 27893)	LPGE	9.6±0.57	10.8±0.94	12.3±0.57	13.7±0.63	6.25
	LPEE	9.2±0.47	10.3±0.63	13.1±0.94	14.2±0.75	3.13

Values are mean±SEM of three experiments in each group. MIC= minimum inhibitory concentration, ATCC= American Type Culture Collection, LPGE=*Laghupanchamula* containing *Gokshura*, LPEE=*Laghupanchamula* containing *Eranda*

migration and proliferation of endothelial cells, leading to neovascularization of connective tissue cells that synthesize extracellular matrices including collagen and of keratinocytes resulting in re-epithelialization of the wounded tissue.[26] In the excision wound model, both extracts showed faster healing compared with the control group. The faster wound contraction rate of the extracts may be due to stimulation of interleukin-8, an inflammatory α-chemokine, which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes, and may increase the gap junctional intracellular communication in fibroblasts and induce a more rapid maturation of granulation tissue.[27] The granulation tissue of the wound is primarily composed of fibroblasts, collagen and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblasts, which start migrating into the wound gap along with the fibrin strands. LPGE and LPEE showed a decrease in days of epithelization and scar area due to an increase in granulation tissue.

Wound infections are most common in developing countries because of poor hygienic conditions. *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa, Streptococcus pneumonia* and *Klebsiella pneumonia,* etc., are some important microorganisms causing wound infection.[28] Presence of bacteria on an ulcer may lead to active infection, with subsequent interference in the process of wound healing. The detrimental effect of bacteria on wound healing results from several mechanisms. Bacteria release a variety of endotoxins, which may reduce the proliferative capacity of fibroblasts and epithelial cells. Moreover, bacteria may affect cell function even to the extent of cellular destruction. Secreted toxins may cause lysis of collagen and fibrin as well as the degradation of growth factors. In addition, consumption of nutrients and oxygen by the invading bacteria at the expense of the newly forming tissue leads to tissue anoxia, with further delay in the healing process.[29] LPGE and LPEE showed antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aerugenosa*, and lowest MIC was found with LPEE against *Staphylococcus aureus*. Beside promoting collagen deposition, both LPGE and LPEE have antimicrobial properties, which may contribute to their early healing potential.

Phytochemical analysis of plants used in LPGE and LPEE reported the presence of flavonoids, tannins, alkaloids, essential oils and saponins.[6-12] Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and have anti-inflammatory and wound healing properties.[30] Alkaloids, flavonoids and saponins have also been reported to possess anti-oxidant, anti-radical, wound healing and antimicrobial properties.[31-34] The wound healing and antimicrobial effects of the extract may therefore be due to the presence of flavonoids, tannins, alkaloids and saponins. The limit test (acute toxicity) study in mice also revealed the safety profile of the test extracts, LPGE and LPEE.

It could be concluded that 50% ethanolic extract of *Laghupanchamula* formulations containing either *Eranda* or *Gokshura* (LPEE and LPGE) possessed wound healing and antimicrobial activities that could be due to the presence of flavonoids, alkaloids, essential oils, saponins and tannins present in it. Further, we did not find any significant difference in their wound healing activity. Therefore, the use of any of these formulations in the traditional *Ayurvedic* system for the treatment of wound healing is justified.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: A review.Int J Low Extrem Wounds 2003;2:25-39.
2. Trikamji J.Charaka Samhita of Agnivesha, Chikitsa Sthana; Abhaya Amalaki Rasayana Pada. 1st ed.Verse 41-57. Varanasi: Chowkhambha Prakashan; 2007.p. 378-9.
3. Sharma PV. Chakradatta of Chakrapanidatta; Jvara Chikitsa. 1st ed.Verse 169. Varanasi: Chowkhambha Orientalia; 1994. p. 26.
4. Trikamji J.Sushruta Samhita of Sushruta, Sootra Sthana; Dravya sangrhniya Adhyaya. 7th ed. Verse 66-67. Varanasi: Chowkhambha Orientalia. 2002. p. 169.
5. Tiwari PV. Kashyap Samhita by Kashyap, Khila Sthana; Antervartani Chikitsha Adhyaya. 1st ed.Verse 83-87. Varanasi: Chowkhambha Vishvabharti; 2008. p. 563.
6. Emmerich RE. Siddhasara of Ravigupta. 1st ed. Verse 2.29. Wiesbaden: Franz Steiner Verlag GmbH; 1980. p. 29.
7. Kumar N, Prakash D, Kumar P. Wound healing activity of *Solanumxanthocarpum* Schrad. andWendl. fruits. Indian J Nat Prod Resources 2010;1:470-5.
8. Asha VV, Pushpangadan P. Preliminary evaluation of the anti-hepatotoxic activity of *Phyllanthus kozhikodianus*, *Phyllanthusmaderaspatensis* and *Solanumindicum*. Fitoterapia 1998;59:255-9.
9. Ghildiyal S, Gautam MK, Goel RK, Joshi VK. Qualitative phytochemical screening and pharmacognostic standardization of *Solanumanguivi* Lam. root. Asian Pac J Trop Biomed2012;2:1-4.
10. Dharmani P, Palit G. Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol 2006;38:95-9.
11. Rahman MM, Gibbons S, Gray AI. Isoflavanones from *Urariapicta* and their antimicrobial activity. Phytochemistry 2007;68:1692-7.
12. Balasubramani SP, Murugan R, Ravikumar K, Venkatasubramanian P. Development of ITS sequence based molecular marker to distinguish, *Tribulusterrestris* L. (Zygophyllaceae) from its adulterants. Fitoterapia 2010;81:503-8.

13. Ilavarasan R, Mallika M, Venkataraman S. Toxicological assessment of *Ricinuscommunis* Linn root extracts. Toxicol Mech Methods 2011;21:246-50.
14. Ghildiyal S, Gautam MK, Joshi VK, Goel RK. Anti-inflammatory activity of two classical formulations of *Laghupanchamula* in rats. J Ayurveda Integr Med 2013;4:23-7.
15. Gautam MK, Purohit V, Agarwal M, Singh A, Goel RK. *Invivo* healing potential of *Aeglemarmelos* in excision, incision, and dead space wound models.Scientific World Journal 2014;2014:740107.
16. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of *in-vivo* wound healing activity of *Bacopamonniera* on different wound model in rats. Biomed Res Int 2013;2013:972028.
17. Gautam MK, Gangwar M, Nath G, Rao CV, Goel RK. *In-vitro* antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murrayapaniculata* Linn. leaves. Asian Pac J Trop Biomed 2012;2:S1660-3.
18. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008;3:163-75.
19. Gautam MK, Singh A, Rao CV, Goel RK. Toxicological evaluation of *Murrayapaniculata* (L.) leaves extract on rodents. Am J Pharm Toxicol 2012;7:62-7.
20. Gautam MK, Goel RK.Toxicological study of *Ocimum sanctum* Linn leaves: Hematological, biochemical, and histopathological studies. J Toxicol 2014;2014:135654.
21. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of *Mimosa pudica*. J Ethnopharmacol 2009;124:311-5.
22. Varoglu E, Seven B, Gumustekin K, Aktas O, Sahin A, Dane S. The effects of vitamin E and selenium on blood flow to experimental skin burns in rats using the ^{133}Xe clearance technique. Central Euro J Med 2010;5:219-23.
23. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, *et al*. Wound bed preparation: A systematic approach to wound management. Wound Repair Regen 2003;11(Suppl 1):S1-28.
24. Süntar IP, Akkol EK, Yalçin FN, Koca U, Keleş H, Yesilada E. Wound healing potential of *Sambucusebulus* L. leaves and isolation of an active component, quercetin 3-O-glucoside. J Ethnopharmacol 2010;129:106-14.
25. Udupa AL, Kulkarni DR, Udupa SL. Effect of *Tridaxprocumbans* extracts on wound healing.Int J Pharmacognosy 1995;33:37-40.
26. Midwood KS, Orend G. The role of tenascin-C in tissue injury and tumorigenesis. J Cell Commun Signal 2009;3:287-310.
27. Moyer KE, Saggers GC, Allison GM, Mackay DR, Ehrlich HP. Effects of interleukin-8 on granulation tissue maturation. J Cell Physiol 2002;193:173-9.
28. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. ClinMicrobiol Rev 2001;14:244-69.
29. Beg S, Swain S, Hasan H, Barkat MA, Hussain MS. Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacogn Rev 2011;5:120-37.
30. Li K, Diao Y, Zhang H, Wang S, Zhang Z, Yu B, *et al*. Tannin extracts from immature fruits of *Terminaliachebula* Fructus Retz. promote cutaneous wound healing in rats. BMC Complement Altern Med 2011;11:86.
31. Ambiga S, Narayanan R, Gowri D, Sukumar D, Madhavan S. Evaluation of wound healing activity of flavonoids from *Ipomoea carnea* Jacq. Anc Sci Life 2007;26:45-51.
32. Sagesaka YM, Uemura T, Suzuki Y, Sugiura T, Yoshida M, Yamaguchi K, *et al*. Antimicrobial and anti-inflammatory actions of tea-leaf saponin. Yakugaku Zasshi 1996;116:238-43.
33. Xing M, Shen F, Liu L, Chen Z, Guo N, Wang X, *et al*. Antimicrobial efficacy of the alkaloid harmaline alone and in combination with chlorhexidinedigluconate against clinical isolates of *Staphylococcus aureus* grown in planktonic and biofilm cultures. Lett Appl Microbiol 2012;54:475-82.
34. Pereira DM, Valentão P, Pereira JA, Andrade PB. Phenolics: From Chemistry to Biology. Molecules 2009;14:2202-11.