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A Preclinical Study on Wound Healing Activity of *Lawsonia ulba* Linn.

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ABSTRACT

Lawsonia ulba Linn., is a reputed medicinal plant used in the treatment of various skin diseases in the Indian system of medicine. The objective of the study presented in this article was to evaluate the wound-healing potential of the ethanolic extract. The study was done on Wistar albino rats using excision, and dead space wounds models. The extract of *Lawsonia ulba* Linn., treated wounds were found to epithelize faster and the rate of wound contraction was significantly increased as compared to control wounds ($p < 0.01$). Wet and dry granulation tissue weights and granulation tissue tensile strength in a dead space wound model also increased at statistically Significant differences at $p < 0.001$ when compared to control. From the results, it may be concluded that, the ethanolic extract of *Lawsonia ulba* Linn. had greater wound healing activity than the nitrofurazone ointment.

Key words: Excision wound, dead space wound, granuloma, phytoconstituents, nitrofurazone ointment

INTRODUCTION

Wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process results in the restoration of anatomic continuity and function (Lazarus *et al.*, 1994). It is a finely orchestrated and overlapping sequence of events involving control of infection, resolution of functional connective matrix, contraction, resurfacing, differentiation and remodeling (Paul and Sharma, 2004). Wounds are generally classified as, wounds without tissue loss (e.g., in surgery) and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions (Paul and Sharma, 2004). Wound healing involves complex series of interactions between different cell types, Cytokine mediators and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation and remodeling (Douglas and Miller Alan, 2003). Wound healing is a process that is fundamentally a connective tissue response; Initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecule which are later remodeled to form a scar (Patil *et al.*, 2009). Wound healing studies

mainly aim to detect various means and factors influencing healing process, so that they could be either used or avoided in clinical practice to alter the healing process favorably. The process of wound healing occurs in four phases: (i) coagulation, which prevents blood loss (ii) inflammation and debridement of wound (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent that accelerates the above process is a promoter of wound healing (Suguna *et al.*, 1996).

A topical preparation of aescin (obtained from horse chestnut) (Guillaume and Padioleau, 1994), Chamomile (Nasemann, 1975), Chaparral (Kay, 1996) and honey (topical application) (Forest, 1982) has been used topically to decrease inflammation and pain and promote healing of minor wounds. Adequate dietary protein is absolutely essential for proper wound healing and tissue levels of the amino acids arginine and glutamine may influence the protein synthesis in wound repair and immune function (Barbul *et al.*, 1983).

Many Ayurvedic herbal plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way. The healing process can be physically monitored by assessing the rate of contraction of the wound. The plant under study, namely *lawsonia ulba* Linn. contains alkaloids, flavonoids, lignins, triterpenoids, fixed oils, fats, proteins and amino acids. protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. *Lawsonia ulba* Linn., belonging to the family of Lythraceae is an evergreen plant. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. Most of the plant parts are used in traditional system of medicine in India. From its leaves a red-orange dye agent is extracted. This agent has an affinity for bonding with proteins and thus is used to dye human body parts (skin, hair, fingernails), as well as leather, silk and wool (Muhammad and Muhammad, 2005; Natarajan *et al.*, 2003). *Lawsonia ulba* Linn., also acts as an anti-fungal and a preservative for leather and cloth. fungal and a preservative for leather and cloth (Bosoglu *et al.*, 1998). *Lawsonia ulba* Linn., leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (Chetty, 2008; Singh *et al.*, 1982). A survey of literature revealed that not much work has been made to study wound healing activity of this plant; hence it was thought worthwhile to investigate the wound healing activity of *Lawsonia ulba* Linn. extract in efficient experimental models of wound in rats.

MATERIALS AND METHODS

Preparation of leaf extract: The fresh leaves of *lawsonia ulba* Linn., were collected locally in month of February 2005 the collected plants were identified with the help of Dr. Anusha Parthiban, Principal, Dhanalakshmi Srinivan college for women, Perambalur, Tamil Nadu, India confirmed with the voucher specimen kept in the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

The fresh leaves were shade dried at room temperature, pulverized by a mechanical grinder, sieved through 40- size sieve mesh. 500 g of fine leaf powder were suspended in 1500 mL of ethanol for 24 h at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No: 1). The filtrate was placed in a water bath to dry at 40°C and the final ethanol free clear residue was used for the study.

Table 1: Preliminary phytochemical screening of the ethanolic extract of *Lawsonia ulba* Linn.

Test/Reagents used	Extract
Alkaloids	+
Reducing sugar	-
Phytosterol	+
Fixed oil and fats	+
Phenolic compounds and tannins	+
Proteins and amino acids	+
Gums and mucilage	-
Flavonoids	+
Lignins	+
Saponins	-
Triterpenoids	+

+: Positive, -: Negative

Qualitative phytochemical evaluation: The plant extract was subjected to qualitative tests by adopting standard procedure for the identification of the phytoconstituents present in it viz., alkaloids, carbohydrates, glycosides, phytosterols, fixed oils, phenolic compounds, proteins, free amino acids, gums, mucilage, flavonoids, terpenoids, lignins and saponins (Vogel, 1971). The results are presented in Table 1.

Animal used for wound healing activity: Wistar albino rats (150-180 g) were used and six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water *ad libitum*.

The study was carried out in CPCSEA registered (Reg. No: 265/CPCSEA) Animal House of Periyar College of Pharmaceutical Sciences, Tiruchirapalli, during the year 2005-2006. It was approved by the IAEC of the above Institution.

Ointment formulation: Two types of ointment formulations were prepared from the extract: 5% w/w, 10% (w/w), where 5 or 10 g of the extract were incorporated in 100 g of simple ointment base British Pharmacopoeia (BP), respectively (Anonymous, 1993). Nitrofurazone ointment (0.2% w/w, Smith Kline-Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

Excision model: Four groups with six animals in each group were anaesthetized with diethyl ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm² full thickness of skin from the depilated area, the wound was left undressed to open environment. Then the drugs, i.e., the reference standard, 0.2% w/w nitrofurazone (NFZ) ointment, simple ointment B.P., *Lawsonia ulba* Linn. extract ointment 5 and 10% w/w were applied once daily till the wound was completely healed. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percentage reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day (Morton and Malone, 1972).

Dead space model: Three groups of wistar albino rats (150-200 g) were used. Dead space wounds were made by implanting, subcutaneously, a 2.5×2.5 cm polypropylene tube beneath the dorsal

paravertebral lumbar skin. Control animals received 2 mL of 1% Carboxy Methyl Cellulose (CMC), orally, while the test groups received *Lawsonia ulba* Linn. (100 and 200 mg kg⁻¹) orally once daily for 10 days. On the 11th post-operative day, the dead space wound was excised. Wet weight was recorded and tensile strength determined (Lee, 1968). The granuloma was dried in an oven at 60°C and the dry weight noted.

Measurement of healing: Tensile strength, the force required to open a healing skin wound, was used to measure healing. The instrument used for this measurement is called tensiometer. It was designed on the same principle as the thread tester used in the textile industry. It consisted of a 6×12 inch board with one post of 4 inch long, fixed on each side of the longer ends. The board was placed at the end of a table. A pulley with a bearing was mounted on the top of one of the posts. An alligator clamp with 1 cm width, was tied on the tip of the post without pulley by a piece of fishing line (20-lb test monofilament) so that the clamp could reach the middle of the board. Another alligator clamp was tied on a piece of fishing line with a 1-L polyethylene bottle tied on the other end. The excised granuloma tissue was then placed on a stack of paper towels that could be adjusted so that the polyethylene bottle was freely hanging in the air. Water added to the polyethylene bottle was weighed and considered as the tensile strength of the wound.

Statistical analysis: Data are expressed as Mean±SEM and subjected to Analysis of Variance (ANOVA) test by comparing with different groups.

RESULTS AND DISCUSSION

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2% w/w), extract ointment 5 and 10% w/w and the control group (i.e.,) simple ointments in the excision wound model are shown in Table 2. Wound contracting ability of the extract ointment was significantly greater (p<0.01) than that of the control as well as reference standard (NFZ ointment). The extract ointment produced complete healing at 16th day and 14th day respectively when 5 and 10% w/w extract ointments were used. The extracted treated wounds were found to epithelialize faster. The rate of wound contraction significantly increased in extract treated wounds

Table 2: Effect of *Lawsonia ulba* Linn., leaf extract and nitrofurazone on excision model

Post wounding days	Wound area (mm ²)			
	Control	Nitrofurazone ointment (0.2% w/w)	Extract ointment (5% w/w each)	Extract ointment (10% w/w each)
0	529±19.3 (0.0)	518±17.4 (0.0)	505±23.0 (0)	518±39.8 (0)
2	467±18.4 (11.7)	417±21.6 (19.4)	363±19.8 (28)	435±14.8 (16)
4	403±21.5 (23.8)	321±29.4* (38.0)	343±18.9* (32)	389±18.6* (24)
6	371±14.6 (29.8)	231±23.4** (55.4)	275±14.3* (45)	297±19.4** (42)
8	313±13.9 (40.8)	173±17.6** (66.7)	160±11.5** (68)	143±9.8** (72)
10	297±14.6 (45.2)	129±11.6** (75.0)	105±8.6** (79)	94±5.9** (81)
12	276±11.9 (47.8)	75±6.9** (85.5)	62±5.4** (87)	43±2.1** (91)
14	259±14.3 (51.0)	34±2.4** (93.4)	32±2.8** (93)	16±1.3** (96)
16	231±16.7 (56.3)	9±0.8** (98.2)	18±0.04* (96)	0.0** (100)
18	211±15.3 (60.1)	0.0** (100.0)	0.0** (100)	-

Values are Mean±S.E of 6 animals in each group. Values in parentheses indicates percentage of wound contraction. *Significant differences at p<0.01 when compared to control. **Significant differences at p<0.001 when compared to control

Table 3: Effect of *Lawsonia ulba* linn., on dead space wound in rats (Means±S.E, n = 6)

Treatment	Wet granuloma weight (mg)	Dry granuloma weight (mg)	Tensile strength (g)
Control	209.8±10.3	32.8±1.9	371.0±19.8
Lawsonia ulba Linn. (5% w/w each)	398.9±18.3*	83.2±5.3*	477.9±31.6*
Lawsonia ulba Linn. (10% w/w each)	488.3±29.9*	102.8±9.9*	579.3±38.2*

*Significant differences at $p < 0.001$ when compared to control

compared with those in the control wounds ($p < 0.001$). The extract *Lawsonia ulba* Linn. at (100 and 200 mg kg^{-1}) produced a significant increase in the wet granuloma tissue as well as in the dry weight. The tensile strength was also found to be increased ($p < 0.001$) in the extract treated groups (Table 3).

Earlier studies reported that effects of *Centella asiatica* extract on dermal wound healing in rats. Plant products have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavonoids (Suguna *et al.*, 1996). Nayak (2006) suggested the presence of triterpenoids which were responsible for the effective wound healing activity of *Cecropia peltata* and *Pentas lanceolata* (Nayak *et al.*, 2006). Earlier studies showed the therapeutic importance from *Lawsonia ulba* Linn., an important medicinal plant. This study involves the preliminary screening, quantitative determination and the qualitative analysis of secondary metabolites of *Lawsonia ulba* Linn., The generated data has provided the basis for its wide use as the therapeutant both in the traditional and folk medicines (Nithya, 2011). Parthiban *et al.* (2011) reported that the bacterial cellulose for the healing of wounds with slight alterations. Generally cellulose from *Acetobacter xylosum* was used for wound healing but in this study cellulose from *Rhizobium* sp. was used.

Khoshid *et al.* (2010) observed that *P. tenuiflorus* leave juice and essential oil had a healing promoting effects using rat wound model. Stimulatory effects were also observed on fibroblast proliferative activity in tissue culture. These effects may be due to the high content of the Ca, Mg, Zn and Ca^{2+} minerals. Improved collagenation was observed in *Memecylon umbellatum* Burm extract ointment treated groups in a dose dependent fashion as compared to groups treated with simple ointment base BP. Ursolic acid was the only component which may be responsible for collagen synthesis stimulation (Puratchikody and Nagalakshmi, 2007). Most of the therapeutic effects of garlic are known to referable to its sulfur-containing compounds Biochemical analysis of garlic extracts in this study were revealed that concentration of two sulfur-containing compounds (Allicin and Methyl sulfonyl methane) which very important in healing accelerating efficacy and antimicrobial potency (Jalali *et al.*, 2008). *Lawsonia ulba* (Lythraceae) has been shown to possess antimicrobial, antifungal and antitubercular activities. Its bark is reported to be useful in jaundice and in the enlargement of the spleen (Nagori and Solanki, 2011). Fei *et al.* (2003) reported that the effectiveness of 15 UMF manuka honey in the treatment of burn wounds in contrast to the control group for wounds tensile strength and histopathological changes during healing process. The treatments were applied on the deep partial thickness burn wounds inflicted by modified electric solder. The wound healing activity of the *Kingelia africana* plant leaf extracts may be due to its angiogenic and mitogenic potential leading to increased cellular proliferation and increased collagen synthesis (Pattanayak and Sunita, 2008). Collagen gives strength and integrity to the tissue matrix and plays a role in homeostasis and epithelialization at latter phase of healing (Hassan *et al.*, 2011).

CONCLUSION

The wound healing property of the leaves extract of *Lawsonia ulba* Linn. appears to be due to the presence of its active principles which accelerates the healing process and confers breaking

strength to the healed wound. The present study has demonstrated that an ethanolic extract of *Lawsonia ulba* Linn., leaves has properties that render it capable of promoting accelerated wound healing activity compared with placebo control. Wound contraction, increased tensile strength activity support further evaluation of *Lawsonia ulba* Linn., in the topical treatment and management of wounds.

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