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Study of wound healing activity of *Delonix regia* flowers in experimental animal models

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ABSTRACT

Delonix regia, a well known plant with high medicinal value, reported to have a number of biological activities including antioxidant, and presence of flavonoids in its chemical constituents. Antioxidant property and flavonoids have been associated with wound healing actions of plants. The present study was done to investigate the wound healing properties of *Delonix regia* in experimental animal models. The ethanolic and aqueous extracts of *Delonix regia* flowers were prepared to study the effect on wound healing in albino rats using incision and excision wound models. Healing was assessed by the rate of wound contraction, period of epithelialisation, tensile strength (skin breaking strength) and estimation the hydroxyproline content. The extracts significantly promoted the healing process, as evident by an increase in wound breaking strength, percentage of wound contraction, increased hydroxyproline content and decreased epithelialisation period, suggesting the possible utilization of this plant to enhance wound healing.

Key words: *Delonix regia*, flowers, extract, wound.

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INTRODUCTION:

Wound healing and tissue repair are complex processes that involve a dynamic series of events including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling[1]. These phases run either concurrently or intimately inter-linked through some chemical, biochemical and cellular pathways. A treatment could influence the healing of wounds by intervening in one or many phases of wound healing. No treatment, either systemic or local, could be considered inert on the healing process unless it is proved experimentally. Wound care and maintenance involve a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical and systemic antimicrobial agents and healing promoting drugs.

Delonix regia (family: Leguminosae, sub-family: Fabaceae) commonly called as *gulmohor* in Hindi. A literature survey[2-7] revealed that *Delonix regia* flowers possess important pharmacological activities like- anti-inflammatory, analgesic, cytotoxic, hepatoprotective, gastroprotective, antidioarrhoeal and antifertility, and anti-fungal activities. The flowers have been used as traditional herbal remedies for gynecological disorders[8]; they are also used as tablet binder[9]. The flowers contain β-sitosterol, alkaloid, flavonoids, carotenoids, phytotoxins and saponins[10-12]. Flower of *Delonix regia*, also contain flavonoids, are used as wound healing agent in households. Recent studies on wound healing activity showed that flavonoids promote significant wound healing property[13].

However, there is no scientific proof justifying the traditional use of *Delonix regia* flower in treatment of wound. Hence we aimed to evaluate its wound healing efficacy in different experimental models of wound healing in rats.

MATERIALS AND METHODS

Plant material

Delonix regia flowers were collected in the month of May-June from the fields of Harapanahalli. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, Karnataka. A herbarium specimen has also been deposited in the college museum (Herbarium specimen no. SCSCOP/P.COL/6/08-09).

Extraction

The flowers were shade dried at room temperature, pulverized and packed in soxhlet column for successive extraction with 70% ethanol for about 48 h. The extract was filtered and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Buchi, Switzerland) and

stored in desiccators until further use. For aqueous extract, flowers were macerated with distilled water (1 Lit.) for three days with intermittent stirring, filtered and concentrated. Both the extracts were subjected to preliminary phytochemical tests.

Preliminary Phytochemical analysis

The preliminary quantitative phytochemical studies were performed for testing the different chemical groups present in the 70% ethanolic and aqueous extract of the flower of *Delonix regia*.

Animals

Healthy albino rats of Wistar strain weighing 150-200 g were used for the study. They were individually housed and maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after experiments. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using anesthetic ether. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Preparation of Ointment

Simple ointment was prepared by taking weighed quantities of white bees wax (2% w/w), hard paraffin (3% w/w), cetosteryl alcohol (5%w/w) and white soft paraffin (90% w/w) and melted. Weighed quantities of 70% ethanol and aqueous flower extract of *Delonix regia* (5% w/w and 10%w/w) were mixed with simple ointment by using ointment slab and spatula.

Wound healing activity

Excision, incision and dead space wound models were used to evaluate the wound healing activity of the plant.

Excision Wound Method

Excision wounds were made as described by Morton and Malone[14] by excising the full thickness circular skin (approx 500 mm^2) from the nape of the neck under ether anaesthesia. The rats were kept individually in separate cages. The animals were divided into six groups comprising six animals in each group. The animals of group I were untreated and considered as the control, group II served as reference standard and treated with 5% w/w povidine iodine ointment, group III & IV treated with ethanolic extract (5% and 10%) and group V & VI treated with aqueous extract (5% & 10%) of *Delonix regia* flowers. Wound closure rate was assessed by tracing the wound on polythene paper on wounding day, followed by 4th, 8th, 12th, 16^{th} day and subsequently on every alternate day/daily till complete closure. Falling of the scab without any raw area indicated time for complete epithelization and the same was noted. Similarly, scars

were traced on complete epithelization to assess wound contraction by noting scar size and shape. Round/oval, large scars indicated poor contraction while stellate shaped or linear scars indicated enhanced wound contraction.

Incision Wound Method

Resutured incision wounds were inflicted with two 6 cm long paravertebral parallel incisions under light ether anaesthesia[15]. These incisions were 1 cm away laterally to the vertebral column. The wounds were closed with sutures made of '0' no. silk thread. The animals were divided into 3 groups each with 6 animals, Group I served as control and treated orally with normal saline. Group II treated with 70% ethanol extract of *Delonix regia* flower orally 250 mg/kg b.w. Group III treated with Aqueous extract of *Delonix regia* flower orally 250 mg/kg b.w. Sutures were removed on 8th day and breaking strength was measured on 11th day post wounding by continuous water flow technique[15]. Three readings were taken on each wound and the mean of six such readings in each animal was used for statistical analysis. This method indirectly estimates the extent of wound healing that is, more the wound tensile strength more is the deposition of collagen in the wound area. Subsequently animals were sacrificed by overdose of anaesthesia.

Dead space wound Method

Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg) and cylindrical grass piths (25 mm x 3 mm) subcutaneously in the groin and axilla randomly by the reported technique[17]. An average, rat consumes 110 mL of water/kg/day, we dissolved 250 mg of flower extract in 100 mL of drinking water. The control group animals were administered with plain drinking water. The granulation tissues were removed on 11th day post wounding. All the granulation tissues were dissected out after sacrificing the animal and were dried at 60°C overnight to record the constant dry weight, expressed as mg/100g body weight. One of the granulation tissues over the grass piths was opened and trimmed to a rectangular piece for estimation of breaking strength and subsequent estimation of hydroxyproline content colorimetrically[18].

Estimation of Hydroxyproline

Dry granulation tissues from both control and treated group were used for the estimation of hydroxyproline[18]. Hydroxyproline present in the neutralized acid hydrolysate were oxidized by sodium peroxide in presence of copper sulfate and subsequently they were complexed with para-dimethylaminobezaldehyde to develop a pink color which was measured at 540 nm by Spectrophotometry.

Histopathological study

For histological studies, granulation tissues were fixed in 10% neutral formalin solution for 24 h and dehydrated with a sequence of ethanol-xylene series of solutions[19,20]. The materials were in filtered and embedded with paraffin (40-60 °C). Microtome sections were taken at 10 µ thickness. The sections were processed in alcohol-xylene series and stained with hematoxylin-eosin dye. The histological changes were observed under a microscope.

Statistical analysis

The results were expressed as mean±standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test and $p < 0.001$ was considered significant.

RESULTS AND DISCUSSION

Delonix regia flowers were collected, identified, authenticated and used for the preparation of 70% ethanolic extract & aqueous extract. The extracts were used for the study.

Preliminary Phytochemical analysis

Preliminary Phytochemical investigation on 70% ethanolic and aqueous extracts of *Delonix regia* flowers showed the presence of carbohydrate, flavonoids, saponin, tannins and steroids.

Wound healing activity

Wound healing activity of the plant was determined in albino rats using excision, incision and dead space wound models.

Excision wound healing

The rate of wound closure in ethanolic and aqueous extract treated animals was significantly more on 4^{th}, 12^{th} & 16^{th} day. However, there was no significant change in rate of wound closure in control group animals. The time for epithelization was 23.52 ± 0.80 days in control group, while it was significantly decreased in the ethanolic and aqueous extract treated group with a mean value of 21.00±0.37 and 19.83±0.30 days (Table 1).

Incision wound healing

The aqueous extract (250 mg/kg b.w.) significantly increased wound breaking strength, 347.28±1.57, compared to that of the control, 119.33±6.67. However, ethanolic extract (250 mg/kg) show lesser significant effect on breaking strength 226.49±3.86 compare to the aqueous extract and control group (Table 2).

Table 1: Effect of 70% Ethanolic and aqueous extract of *Delonix regia* flower on Excision wound parameters

Group	% wound contraction on 4^{th} day	8^{th} day	12^{th} day	16^{th} day	Epithelization time (Days)
Control	13.60±0.52	35.44±1.26	51.20±1.38	68.00±0.88	23.52 ± 0.80
Standard	35.08±1.62***	57.72±1.94***	86.40±0.77***	97.84±0.29***	18.00±0.87***
5% Ethanol	27.28±1.86*	49.32±1.59	66.16±0.85	76.28±0.68*	22.71±0.43*
10% Ethanol	36.72±0.83***	58.00±1.15*	69.92±0.56*	81.48±0.83**	21.00±0.37*
5% Aqueous	24.52±0.80*	55.88±1.10*	69.04±1.16*	87.28±0.73**	20.35±0.25*
10% Aqueous	34.00±1.09***	61.28±1.09***	73.00±0.65***	93.08±0.60***	19.83±0.30**

The values are expressed as Mean ± SEM, n=6 in each group.
*P<0.05, **P<0.01 and ***P<0.001 vs. control

Table 2: Effect of 70% Ethanolic and Aqueous extract of *Delonix regia* flower on Incision and dead space wound model.

Parameters	Control	Ethanolic extract	Aqueous extract
Incision wound model (250 mg/kg, once a day)			
Tensile breaking strength	119.33 ± 6.67	226.49 ± 3.86***	347.28 ± 1.57***
Dead space wound model (250 mg of flower extract in 100 ml water)			
Tensile breaking strength	183.79±0.88	238.80±25.213	431.802±2.54**
Wet granulation tissue weight (mg/100 gm b.wt.)	191.7±0.58	213.8±0.85	294.4±0.99***
Dry granulation tissue weight (mg/100 gm b.wt.)	28.1±0.43	40.4±0.88	50.1±0.85***
Hydroxiproline (mg/gm tissue)	21.504±1.13	34.424±1.17	51.878±1.34***

The values are expressed as Mean ± SEM, n=6 in each group.
*P<0.05, **P<0.01 and ***P<0.001 vs. control.

Dead space wound Method

The aqueous extract increased breaking strength (347.28±1.57) of granulation tissue significantly, similar to its effects in resutured incision wounds. Breaking strength of the granulation tissue in the ethanolic extract treated group (226.49 ± 3.86) did not significantly differ from that of the control (Table 2). Cotton pellet granuloma weight was increased significantly in the aqueous extract treated group (50.1±0.85) as compared to that of control (28.1±0.43),while granuloma dry weight in ethanolic extract treated group (40.4±0.88) also significant from that of the control. Hydroxyproline content was significantly increased in aqueous extract treated animals (51.878±1.34) in comparison to that of the control (21.504±1.13) (Table 2).

Histopathological study

Granulation tissues were used for histopathological study. The tissues were fixed following different steps, embedded with paraffin and microtome sections were taken at 10 µ thickness. The sections of granulation tissue of extracts treated rats showed the sign of tissue repair

(Figures 1-3) as compared to that of the control group. Control group showed well formed but thick granular cell layer and the underlying dermis contained deposited collagen fibers with inflammation (Figure 1). The animals treated with ethanolic extract of *Delonix regia* showed thin epidermis with hair follicle formation in the dermis and some inflammatory cells in a well organized dermis (Figure 2).

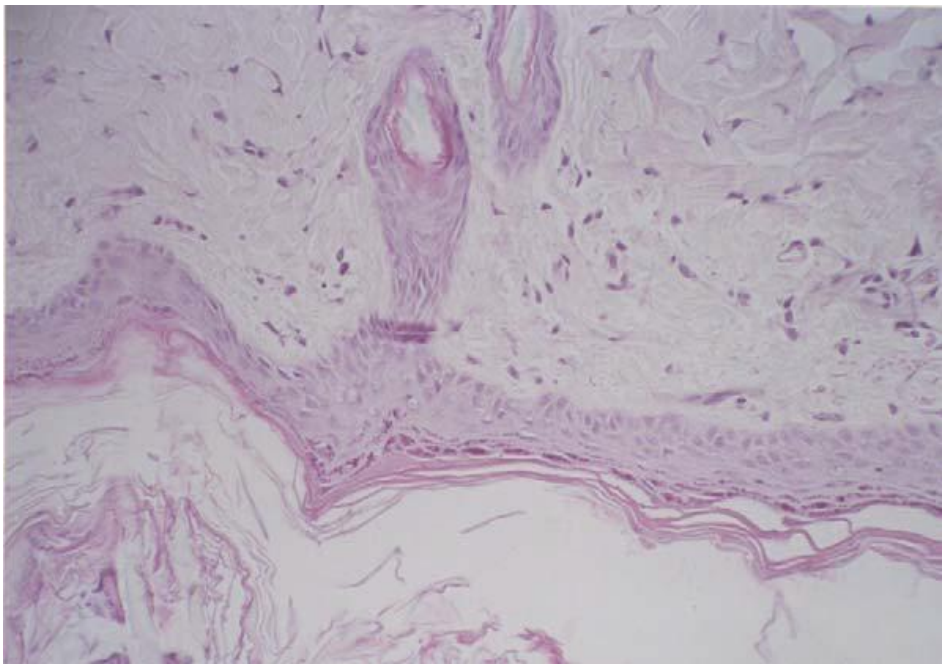

Figure 1: Control group showing well formed but thick granular cell layer, the underlying dermis contains deposited collagen fibers with inflammation.

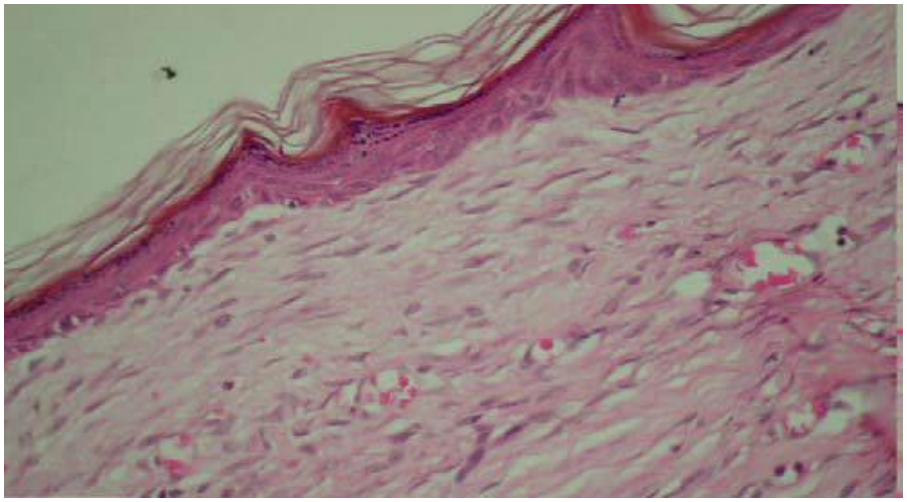

Figure 2: Animals treated with ethanolic extract of *Delonix regia* showing thin epidermis with hair follicle formation in the dermis with some inflammatory cells in a well organized dermis.

The aqueous extract treated animals showed thin well-formed epidermis with hair follicle formation in the dermis and no inflammatory cells in a well organized dermis (Figure 3).

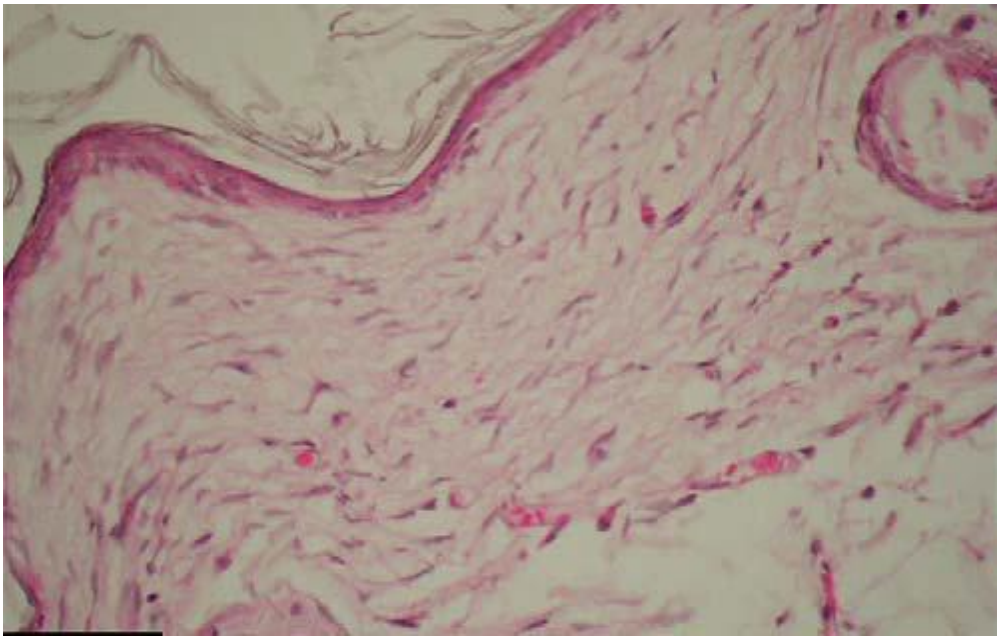

Figure 3: Animals treated with aqueous extract of *Delonix regia* showing thin well-formed epidermis with hair follicle formation in the dermis and no inflammatory cells in a well organized dermis.

Wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. The granulation tissue of the wound is primarily composed of fibroblasts, collagen, edema, and new small 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. The collagen, composed of the amino acid, hydroxyproline, is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index for collagen turnover.

Hence in the present study three different wound models were used to determine the healing actions of *Delonix regia* flowers. In the incision wound model, a significant increase was observed in the skin tensile strength of the aqueous and ethanol extract- treated group, at both dose levels (Table 2). The drug-treated animals of the dead-space wound model showed a significant increase in dry granuloma weight, granuloma breaking strength and the level of hydroxyproline content (Table 2). The histopathological study revealed increased collagen deposition in the drug, treated group (Figures 1, 2), as compared to that of the control.

In studies using the excision wound model, animals treated with the ethanol extract of *Delonix regia* showed a significant decrease in the epithelization period, as evidenced by the shorter period for the fall of eschar compared to control. The drug extract also facilitated the rate of wound contraction significantly at both dose levels (Table 1).

In present investigation, preliminary phytochemical analysis of ethanolic and aqueous flower extract revealed the presence of flavonoids, saponins, tannins and carbohydrates. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis[16, 22].

Further, tannins promote the wound healing through several cellular mechanisms; chelation of free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation capillary vessels and fibroblasts and including keratinocyte proliferation, but do not act on the differentiation towards cornified cells[21,23].

However, our results revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property.

Therefore, it can be confirmed that, in present investigation significant wound healing potential of *Delonix regia* flower may be due to flavonoids and tannins content, which were confirmed by preliminary phytochemical screening.

The present study thus demonstrated that an ethanol and aqueous extract of *Delonix regia* possessed wound healing activity comparable to that of standard drug and control. Wound contraction and increased Hydroxyproline observed in the present work provide scientific evidence support the usage of the plant extract in the topical treatment and management of wounds.

CONCLUSION

In conclusion, the results of the present study shows the wound healing properties of *Delonix regia* flowers extracts in terms of faster rate of wound closure and epithelization period in excision wound model and significant increase in strength in incision wound model. Therefore, the findings from the present work justify the wound healing activity of *Delonix regia*. However, it is difficult to say which component(s) of the extract are responsible for the wound healing activity and further phytochemical studies are required to isolate the active compound(s) responsible for the activity.

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