WOUND HEALING ACTIVITY OF WEDELIA CHINENSIS LEAVES

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Summary

The wound healing efficacy of ethanolic leaf extract of *Wedelia chinensis* was evaluated in excision, incision and dead space wound models. The parameters studied include rate of wound contraction, period of complete epithelialization, tensile strength of incision wound and, granulation tissue dry weight. Its ethanolic extract was found to possess significant wound healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight, and its breaking strength.

Key Words: *Wedelia chinensis*, wound healing, excision wound model, incision wound model.

Introduction

The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners (1). Natural products are a source of synthetic and traditional herbal medicines which are still the primary health care system in some parts of the world (2). The past decade has seen considerable change in opinion regarding ethnopharmacological therapeutic applications. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties.

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling.

The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts exert collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells.

Wedelia chinensis Merrill (Syn. *Wedelia calendulaceae*) (Asteraceae) is a small much branched annual herb, commonly known as "Pilabhamgara" or "Bhringraj'in Hindi and is a reputed herbal medicine in both Ayurvedic and Unani system of medicine. The herb contains wedelolactone and demethylwedelolactone (Coumestans derivatives) possessing potent anti-hepatotoxic effect and is incorporated as a major ingredient in a number of developed potent anti-hepatotoxic phytopharmaceuticals formulations. It is useful in the treatment of osteoporosis of knee and also possesses anti-inflammatory activity (3,4,5). As it contains large amount of phenolic constituents and it is also effective in the treatment of inflammatory conditions, so its wound healing activity was studied in details.

Extracts from the dried or fresh leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the leaves of *Wedelia chinensis* has been used by Ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne. Some work on the wound healing activity of the aqueous extract of the leaves of this plant on open wound and sutured wound models is already on record (6). The present investigation confirms the above finding on its alcoholic extract and in addition reports the study using dead space wound model and estimation of hydroxyproline content in the granulation tissue which suggests the increase in the collagen turnover during the process of wound healing.

Material and methods

Plant material: The whole plant of *Wedelia chinensis* was procured from the Plant Physiology Division, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, M.P. and authenticated by the taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi. A voucher specimen (**NHCP/NBPGR/2007/99/2225 dated 22/08/2007**) was retained in our laboratory for further reference.

Plant extract: The plant material was dried under shade, reduced to moderately coarse powder and was extracted successively with petroleum ether (60-80^{o}C), ethanol in a soxhlet apparatus. The ethanolic extract was dried under vacuum (yield 6.78%), and its qualitative analysis showed the presence of phenolic compounds (including flavonoids) saponins and reducing sugars. The ethanolic extract of *Wedelia chinensis* was used for the wound healing studies.

Animals: The Institutional Animal Ethics Committee, (IAEC) approved the use of animals for the present study**, (Ethical clearance number: 711/02/a/CPCSEA).**

Healthy Wistar albino rats of both sexes 200–220 g was used for the study. They were individually housed and were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. Animals were periodically weighed before and after the experiment. The rats were anaesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anaesthesia (120 mg/kg). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

Wound-healing activity: Excision, incision and dead space wound models were used to evaluate the wound-healing activity of ethanolic and aqueous extracts of *Wedelia chinensis.*

Excision wound model: Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malon (7). The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5 cm (circular area = 300mm^2) in length and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open (8). The animals were divided into two groups of 6 each. Group 1 animals were topically treated with the simple ointment base I.P. (Indian Pharmacopoeia 1966) as a placebo control. The animals of group 2 were topically treated with the 10% ointment of the ethanolic extract of *Wedelia chinensis* formulated in simple ointment base I.P. (Indian Pharmacopoeia 1966) till complete epithelization (9). The wound closure rate was assessed by tracing the wound on days 1, 5 and 15 post-wounding using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

Incision wound model: As with the above model rats were anaesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimeters in length was made through the skin and cutaneous muscle on the back as described by Ehrlich and Hunt et al. (10). After the incision, surgical sutures were applied to the parted skin at intervals of one centimeter. The wounds were left undressed. The rats were given *Wedelia chinensis* extract (dissolved in tween- 80, 0.5%) orally at a dose of 500 mg kg^{-1} day^{-1}. The controls were given with tween-80 0.5% only. The sutures were removed on the 8th post wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method described by Lee (11).

Dead space wound model: Dead space wounds were inflicted by implanting two sterilized cotton pellets (10 mg), one on either side of in the lumbar region on the ventral surface of each rat. On the 10th postwounding day, the granulation tissue formed on the implanted cotton pellet was carefully removed. The wet weight of the granulation tissue was noted. These granulation tissues were dried at 60°C for 12 hours, and weighed, and the weight was recorded. To the dried tissue added 5 ml 6 N HCl and kept at 110°C for 24 hours. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline (12).

Determination of wound breaking strength: The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound, and the procedure was repeated on the contralateral wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Estimation of Hydroxyproline: Hydroxyproline present in the acid hydrolysate of granulation tissue oxidized by sodium peroxide in the presence of copper sulfate, when complexed with para-dimethylaminobezaldehyde, develops a pink color that was measured at 540 nm using colorimetry.

Results

The significant increase in the wound-healing activity was observed in the animals treated with the *Wedelia chinensis* extract compared with those who received the placebo control treatments. Table 1 shows the effects of the ethanolic extract *Wedelia chinensis* administered orally at a dose of 500 mg kg^{-1} day^{-1} for 10 days on wound healing activity in rats inflicted with incision wound. In the incision wound model, a significant increase in the wound breaking strength (451.01 ± 0.1249g) was observed when compared with the controls.

Table 1: Wound healing effect of *Wedelia chinesis* in Incision wound model

	Tensile strength(g)	
Parameter	**Placebo control**	**Experimental**
Skin breaking strength (g)	325.66 ± 0.1282	451.01 ± 0.1249*

n = 6, Values are expressed as mean ± SEM
*p<0.001 significant as compared to control.

In the excision wound model, *Wedelia chinensis* treated animals showed a significant reduction in the wound area ($p < 0.001$) and epithelization period (Table 2). In the dead space wound model (Table 3); the ethanol extract-treated animals showed significantly increased levels of hydroxyproline content ($p<0.001$) as compared with the control group of animals. A significant increase was observed in the weight ($p < 0.001$) of the granulation tissue in the animals treated with the extract.

Table 2: Wound healing effect of *Wedelia chinesis* in Excision wound model

Parameter	**Placebo control**	**Experimental**
Wound area (mm^2)		
Day 1	249.66 ± 1.5423	250.83 ± 1.7016
Day 5	196.16 ± 1.1379	180.83 ± 1.0139*
Day 15	148.5 ± 0.8467	80.33 ± 1.1157*
Period of epithelization (day)	19.0 ± 0.3652	15.1 ± 0.3073*

n = 6, Values are expressed as mean ± SEM
*p<0.001 significant as compared to control.

Table 3: Wound healing effect of *Wedelia chinesis* in Dead space wound model

Parameter	**Placebo control**	**Experimental**
Wet weight of the granulation tissue (mg/100 g rat)	80.90 ± 0.1770	99.65 ± 0.2432*
Dry weight of the granulation tissue (mg/100 g rat)	7.93 ± 0.1977	11.81 ± 0.1922*
Hydroxyproline (mg/g tissue)	20.58 ± 0.4045	41.83 ± 0.3861*

n = 6, Values are expressed as mean ± SEM
*p<0.001 significant as compared to control.

Discussion

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. It has 3 phases; inflammatory, proliferative and maturational and is dependent upon the type and extent of damage, the general state of the host's health and the ability of the tissue to repair. The inflammatory phase is characterized by hemostasis and inflammation, followed by epithelization, angiogenesis, and collagen deposition in the proliferative phase. In the maturational phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content. The ethanolic extract of *Wedelia chinensis* demonstrated a significant increase in the hydroxyproline content of the granulation tissue indicating increased collagen turnover. Collagen, the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen (13).

The wound-healing property of *Wedelia chinensis* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the incision wound observed in our study may have been contributed by the tannin phytoconstituent of *Wedelia chinensis* from the astringent effect which has been reported elsewhere (14) Further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

Conclusion

The present study has demonstrated that the ethanolic extract of *Wedelia chinensis* leaves has properties that render it capable of promoting accelerated wound healing activity compared with placebo controls. Wound contraction, increased tensile strength and increased in hydroxyproline content support further evaluation of *Wedelia chinensis* in the topical treatment and management of wounds.

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