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Evaluation of Wound Healing Potentiality of Methanolic Extract of *Wedelia chinensis* Whole Plant

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ABSTRACT

The methanolic extract of *Wedelia chinensis* (Asteraceae) whole plant was evaluated for its efficacy against wound healing activity by excision wound healing models using Albino wistar rat. In this study whole plant parts powder were extracted using methanol, it was used further for evaluation of wound healing activity. Wound healing activity was assessed by observing the following parameters, period of epithelialization and measuring the area of wound contraction for excision model. The methanolic extract of the whole plant of *Wedelia chinensis* showed the significant wound healing activity on excision wound healing model which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction. Therefore the present investigation revealed the traditional claim of *Wedelia chinensis* as a wound healing drug in the Indian system of medicine.

Keywords: *Wedelia chinensis*, Exicison model, methanolic extract, Epithelialisation.

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INTRODUCTION

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 remodelling. 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[1].

The basic principle of optimal wound healing is to minimise tissue damage and provide adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part[2]. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to repair and remodelling of the damaged tissue[3]. Presence of zinc helps in wound healing and is good for protein metabolism. It is beneficial for the development of the reproductive system. Zinc helps maintain collagen, which keeps the skin smooth, supple and firm[4].

Wedelia chinensis (Asteraceae) is a perennial herb of about 0.3 to 0.9 cm height. Leaves are fleshy, usually 4-9 cm long and 2-5 cm wide, irregularly toothed or serrate, usually with a pair of lateral lobes and obviate in shape. Flowers are yellow, tubular in terminal or axillary head and 4-5 cm in diameter. Traditionally the fruits, leaves and stem are used in childbirth and in the treatment of bites and stings, fever and infection. The leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhea[5]. The leaves are also used for dyeing hair and for promoting their growth. The tonic of the leaves is used in cough and cephalalgia. Decoction of the plant is used in menorrhagia and skin diseases[6]. The plant has also found its use in inflammations, helmentic diseases and liver disorders [7].The decoction of the plant was extensively used by the tribes in Kolli Hills of Namakkal District, Tamilnadu, India, to reduce mental tension and also to induce sleep and the plant affects CNS [8].The plant has been used as astringent, bitter, acrid, anti-inflammatory, cardiotonic, treatment of wounds, seminal weakness and viral-hepatitis[9]. A methanolic (5 %) extract inhibits the growth of Ehrlich's ascites carcinoma. The extracts of this plant have been tested in experimental animal models for their hepatoprotective effect [10], analgesic and anti-inflammatory activity and androgen suppressing activity [11].The alcoholic extract of the leaves was found to possess wound healing properties [12], antioxidant [13] and lipid peroxidation inhibitory activity [14] in rats. The plant is traditionally used to reduce mental tension and to induce sleep and scientifically reported to possess antioxidant property which indicates its usefulness in reducing anxiety and stress in emotional conditions. Therefore, in the light of the traditional and reported uses, the present study was undertaken to investigate the wound healing activity of the ethanol extract

of *Wedelia chinensis* whole plant in various experimental animal models.

MATERIAL AND METHODS

Plant material

Whole plants of *Wedelia chinensis* were collected in the month of August, 2012 from the college campus, Noida Institute of Engineering and Technology, Knowledge Park II, Greater Noida, UP, India. The plant were authenticated by Dr. Roshini Nayar, National Bureau of Plant Genetic Resources(Indian Council of Agriculture Research), Pusa campus, New Delhi-110012 and the voucher specimen NHCP/NBPGR2012-29 is preserved in the Pharmacognosy Department of our Institute for further reference. The plants were dried under shade with occasional shifting and then powdered with mechanical grinder and stored in an air tight container for further extraction purpose.

Preparation of extract

The powered material (whole plant) was subjected to hot continuous soxhlet extraction with methanol (35-45°C) for 72 hours. The filtrate was collected and marc was again subjected to extraction process for further 24 hours. The filtrates were collected and were concentrated over water bath maintaining a temperature of 40°C .The concentrated mass was cooled and finally it was placed in the desiccators and was used for further studies. The percentage yield of the extract was calculated as 23.0%w/w. The preliminary phytochemical screening was carried out and it shows the presence of the following constituents like alkaloid, coumarins, flavone, steroid, tannin, glycoside/sugar, terpenoid and saponins.

Animals

Albino rats (Wistar) weighing 150- 200g of either sex were used for the study. They were procured from the animal house of NIET, Greater Noida. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27C less than 12 hours dark/ light cycles. They were fed with standard rat feed and water *ad libitum* was provided. The litter in the cages was renewed thrice a week to ensure hygenicity and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animal Ethical Committee prior to the beginning of the project work bearing the protocol number NIET\IAEC\2013\46.

Method:

Exicion wound model

The experimental animals were grouped in to four containing six animals each and treated as follows:

Group I: Received control
Group II: Received Neosporin ointment
Group III: Received test ointment, which is made up by methanolic extract of whole plant of *Wedelia chinensis* (5% w/w)
Group IV: Received test ointment, which is made up by methanolic extract of whole plant of *Wedelia chinensis* (10% w/w)

A circular wound of about 500sq mm full thickness of a predetermined area was made on the depilated back of the rat. The ointment was topically applied once a day, starting for the day of wound made, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wound were traced on mm^2 graph paper on days 3, 6, 9, 12, 15 and 18 and thereafter on alternate days until healing was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

Statistical analysis

The experimental results were expressed as multiple comparisons of Mean ± SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparison Test and the values of $*[P < 0.05]$ and $**[P < 0.005]$ were considered statistically significant.

RESULTS

In exicision wound model, the mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15, and 18 post wounding days. The period of epithelialisation 23±0.33 was found to be high in control group which was untreated and take more days in healing while in standard group it was significantly reduced to 18±2.52 of standard drug Neosporin. The period of epithelialisation of test ointment of (5%w/w) and 910%w/w) methanolic extract of whole plant of *Wedelia chinensis* was found to be 20±1.15 and 18±1.89 respectively.

From the experimental data, the 10%w/w B test ointment was more potent than (5%w/w) A test ointment as in B, the wound were traced on mm^2 graph paper on day 3, 6, 9, 12, 13, 15 and on 18 day. The wound was (100%) completely healed, without any residual raw wound. In A test ointment was also showed healing effect but less effective than B test ointment. As the wound were traced on mm^2 graph paper on days 3, 6, 9, 12, 15 and on 18 day, the wound was (98.99%) closed which was more effective than control group.

DISCUSSION

The result of the present investigations revealed that the methanolic extract of plant *Wedelia chinensis* possess significant wound healing activity in the excision wound models. In spite of tremendous development in the field of synthetic drugs during recent era, they are

found to have some or other side effects. Therefore a systematic approach is made to find out the efficacy of plants against wounds so as to exploit them as herbal wound healing agents.

Table 1: Effect of methanolic extract of whole plants of *Wedelia chinensis* on excision wound model

Post wounding day	Wound area (mm^2)±SEM and (% Wound contraction) on day			
	Control (White petroleum jelly)	Neosporin (0.2%w/w)	MEWC (5%w/w)	MEWC (10%w/w)
0	488.5±3.54	480.5±4.193	495.5±4.133	497.5±3.819
3	466±1.23** (4.60)	363.16±2.53** (26.18)	416.33±1.21** (15.97)	426±2.23** (14.37)
6	361.16±2.05* (25.99)	305.10±1.21* (36.50)	352.83±2.35* (28.79)	347.5±2.61** (30.15)
9	312.83±2.53** (35.96)	277±0.96** (42.35)	259.16±1.62** (47.69)	250±3.77** (49.74)
12	227.5±1.25** (53..42)	141.83±0.94** (70.48)	114.5±0.95* (76.89)	91.33±1.14** (81.64)
15	150±1.76** (69.29)	25.16±0.83** (94.76)	34±1.29** (93.18)	10.5±0.88** (97.88)
18	66.83±1.55** (86.31)	0	5±0.73** (98.99)	0
Epithelialisation Time (days)	23±033	18±2.52**	20±1.15*	18±1.89**

Figures in parenthesis indicates % of wound contraction

***P<0.05,*P<0.01, when compared with control group. One way Anova followed by Dunnet multiple comparison test.*

Experimental assessment of the wound healing activity of methanolic extract showed increased rate of wound contraction and epithelialization in treated animals. Topical application of the methanolic extract on excision wounds accelerated wound contraction and reduced wound epithelialization period in rats. Wound healing involves regeneration of specialized cells by proliferation of surviving cells and connective tissue response characterized by the formation of granulation tissue[15]. It is also characterized by haemostasis, reepithelialisation and remodelling of the extra cellular matrix. Epithelialization which is the process of epithelial renewal after injury, involves the proliferation of and migration of epithelial cell towards the centre of the wound while wound contraction is largely due to the action of mayofibroblasts[16,17]. Thus the effect of methanolic extract of the acetone fraction on wound contraction and epithelialization suggest it may enhance epithelial cells migration and proliferation, as well as the formation, migration and migration of mayofibroblast. On chronic oral administration, methanolic extract and acetone fraction enhanced the granuloma tissue formation in dead space wounds. Granuloma tissue formed on an inert foreign body in a dead space comprises an accumulation of modified macrophages[15], histological giant cells an undifferentiated connective tissue consisting largely of collagen[15,17,18]. Increase in granuloma tissue in dead space wound is associated with enhanced collagen maturation and increased protein content as well as angiogenesis[19,20,21]. These processes are indicator of new tissues generation and suggest the methanolic extract may stimulate mechanisms associated with tissue regeneration. Closely related to this is the effect of growth factors

secreted by macrophages on wounds. Macrophages secrete peptide growth factors that exert preheating effect by stimulating regeneration, fibroblast proliferation and activation and angiogenesis[15]. It is, therefore, likely that in addition to enhancing wound contraction and epithelialization, the methanolic extract may also stimulate processes associate with tissue regeneration.

CONCLUSIONS

The methanolic extract of *Wedelia chinensis* (whole plant) showed wound healing property. We propose that the additive and synergistic activity of phytochemicals such as flavonoid, steroid ,tanins and alkaloids present in the methanolic extract of *Wedelia chinensis* were responsible for its potent wound healing property. The present investigation offers scientific evidence to the folkloric accounts of the use of whole plant in treating cuts and wounds.

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