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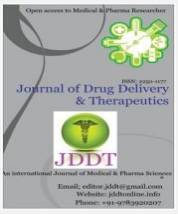

Open Access Research Article

Wound Healing potential of Grandiflorenic Acid Isolated from *Wedelia trilobata* Linn.

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ABSTRACT

The ethyl acetate fraction from ethanolic extract of *W. trilobata* leaves displayed antibacterial and fibroblast stimulatory activities thereby suggesting potential wound healing properties. Ethyl acetate fraction was further subjected to bioassay guided fractionation, which afforded isolation of grandiflorenic acid (GA). GA exhibited potential *in vitro* wound healing activity due to fibroblast stimulation and inhibiting inflammatory phase of wound healing, evident by reduced levels of inflammatory cytokines from macrophage RAW 264.7 cells. The aim of the present study was to evaluate wound healing activity of GA formulated in ointment base (0.5% and 1.0% w/w) using excision, incision and dead space wound models in experimental rats. Treatment of wound with isolated grandiflorenic acid 1.0% w/w topically exhibited significant ($p<0.01$) wound healing activity in all three models as compared to control groups. High rate of wound contraction, decrease in period of epithelialisation, high tensile strength, increase in dry granulation tissue weight.

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INTRODUCTION:

Wound care is often complex, frequently time-consuming, sometimes confusing and nearly always expensive. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered. Medicinal plants have been used since immemorial time for the treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. This revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safe, and clinically effective, better tolerated by patients, less expensive and globally competitive.

Wedelia is seen at its best in tropical climate, where heat and humidity combine to help it produce great sheet of foliage starred with golden daisy flowers. *Wedelia trilobata* contains diterpene (kaurenoic acid), eudesmanolide lactones and luteolin (in leaves and stems). Kaurenoic acid has antibacterial, larvicidal and trypanocidal activity; it is also a potent stimulator of uterine contractions[1]. The ethyl acetate fraction from ethanolic extract of *W. trilobata* leaves displayed antibacterial and fibroblast stimulatory activities thereby suggesting potential wound healing properties [2]. The ethyl acetate fraction was further subjected to bioassay guided fractionation which afforded isolation of GA [3]. GA exhibited potential *in vitro* wound healing activity due to combination of fibroblast stimulation and inhibiting prolonging inflammatory phase of wound healing evident by reduced levels of inflammatory cytokines from macrophage RAW 264.7 cells [4]. The aim of the present study was to evaluate wound healing activity of GA formulated in ointment base (0.5% and 1.0% w/w) using excision, incision and dead space wound models in experimental rats.

MATERIAL AND METHODS:

GA was isolated from leaves of *W. trilobata* in the previous studies [2]. The ointment of grandiflorenic acid (0.5 and 1% w/w) was prepared (Table 1) and stored in airtight container in 4˚C.

Evaluation of Grandiflorenic acid ointment

Physical parameters of cream formulation such as pH, consistency, spreadability and extrudability were determined. Stability studies were carried out following ICH guidelines, to ensure that formulation is stable during different storage conditions.

Table 1: *Formulation of grandiflorenic acid in excision wound model*

S.No.	Ingredient	Percent (w/w)	Percent (w/w)
1.	Mineral oil	10	10
2.	White petrolatum	30	30
3.	Glyceryl monosterate	10	10
4.	Cetyl alcohol	5	5
5.	Glycerin	5	5
6.	Potassium sorbate	0.1	0.1
7.	Grandiflorenic acid	0.5	1
8.	Purified water	39.4	38.9

Animal grouping and treatment

The Wistar rats of either sex weighing 150-200 g were used. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). Wistar rats were divided into four groups consisting of six animals. Group I: Negative control (ointment without GA); Group II: Positive control (povidone iodine ointment); Group III: GA Ointment (0.5%); Group IV: GA Ointment (1%)

Excision wound

A full thickness of the excision wound of circular area (approximately 600 mm^2) was made on the shaved back of the anesthetized rats. The treatment was started as per given treatment schedule. The wounds were monitored and the area of wound was measured on 0, 3, 6, 9, 12 and 15^{th} post-wounding days. The period of epithelialization was calculated as the number of days required for falling of the dead tissue without any residual raw wound.

Incision wound

An incision wound of about 6 cm in length and about 2 mm in depth were made with sterile scalpel on the shaved back of the anesthetized rats. The parted skin was stitched with sterilized needle at 0.5 cm intervals. The wounds of animals in the different groups were treated as per treatment schedule for the period of 10 days. When wounds were cured thoroughly, the sutures were removed on the post-wounding day and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 11^{th} day. Thereafter, the animals were euthanized and the tissues were processed for histopathological examination.

Dead space wound model

Dead space wounds were created by subcutaneous implantation of sterilized cotton piths (10 mg) on the right side groin and axilla on the ventral surface of each rat. The granulation tissues formed on the cotton piths were excised carefully on the 10^{th} post wounding day under light ether anesthesia. The tissue was dried overnight at 60°C and the dried granulation tissue weight was recorded on the 11^{th} day. The granulation tissue so harvested was subjected to hydroxyproline estimation.

Statistical analysis

Data are expressed as a mean ± s.d. Statistical evaluation was carried out using one-way ANOVA followed by Tukey's test. The values of $p < 0.05$ were considered to be statistically significant.

Excision wound study

The wound healing contracting ability of animals treated with GA 0.5% and 1.0% topically was found to be significantly higher ($p < 0.05$) on day 12 and 15 as compared to the control (Table 2). The epithelialization period (complete healing) was also found to be 22.3 ± 1.2 and 20.3 ± 0.9 days in case of animals treated with GA topically, 0.5% and 1.0% respectively.

Table 2: *Effect of grandiflorenic acid in excision wound model*

Treatment & Doses	*Percentage wound contraction (mean ± SEM)*					*Period of epithelization (days)*
	Day 3	*Day 6*	*Day 9*	*Day 12*	*Day 15*	
NC	*25.2 ±1.6*	*24.0±1.5*	*21.7±1.2*	*19.3±0.9*	*16.2±1.0*	*26.2±1.8*
PC (Povidone iodine)	*22.5 ± 0.8*	*18.8±0.9*	*13.8±0.7*	*9.0 ±0.5[a]*	*5.2 ±0.6[a]*	*19.0±1.7[a]*
GA (0.5% w/w)	*24.2 ±1.2*	*21.8±1.0*	*19.0±0.9*	*15.3±0.8*	*10.3±0.9[a]*	*22.3±1.2[a]*
GA (1% w/w)	*23.2 ±1.0*	*19.8±0.9*	*16.2±0.9*	*10.7±0.7[a]*	*5.3±0.6[a]*	*20.3 ±0.9[a]*

NC: Negative control; PC: Positive control, Values are mean ± s.d.,(n=6) . Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test. [a]p<0.05 with negative control

Incision wound study

GA 1.0% topical, significantly increased ($p<0.05$) the tensile strength on 10th post wounding day (501 ± 3.4 g respectively when compared to control (245.5 ± 2.9 g) (Table 3).

Dead space wound study

The groups treated with GA 1.0% w/w topical, significantly increased weight of granuloma by 67.4 ± 0.9 mg/100g, respectively compared to control 30.1 ± 0.9 mg/100g (Table 3).

Table 3 *Effect of grandiflorenic acid in incision and dead space wound model*

Treatment	Incision wound	Dead space wound	
	Tensile strength on 10th day (g)	Granuloma wt. (mg/100 g)	Hydroxyproline content (µg/mL)
NC	245.5 ± 2.9	30.1 ± 0.9	1.5 ± 0.1
PC (Povidone-iodine)	612.5 ± 4.1[a]	74.5 ± 0.8[a]	5.1 ± 0.2[a]
GA (0.5% w/w)	463.9 ± 3.2[a]	54.5 ± 1.1[a]	2.9 ± 0.2[a]
GA (1.0% w/w/)	501 ± 3.4[a]	67.4 ± 0.9[a]	4.3 ± 0.2[a]

Values are mean ± s.d., (n=6). Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.
[a]$p<0.05$ with negative control

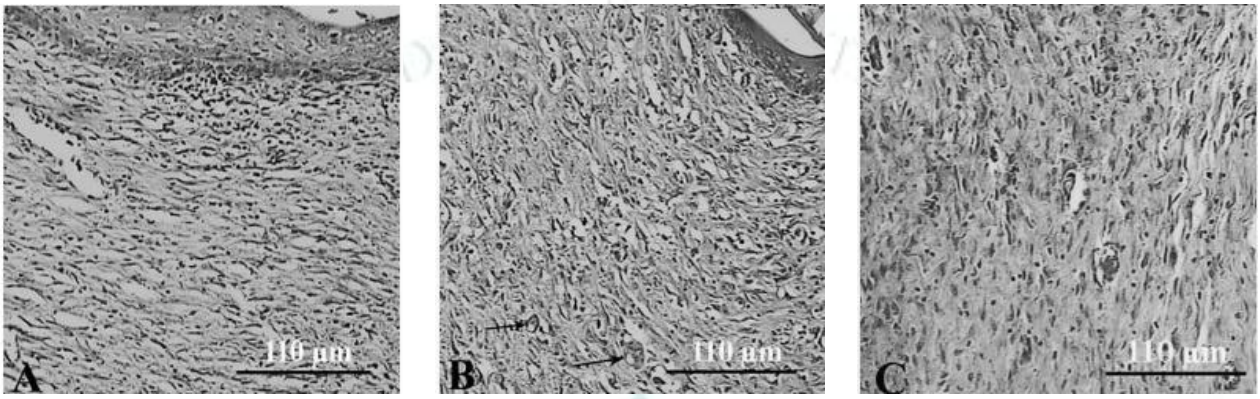

Figure 1: Histological examination (H & E stains, 40X) of the healed wound section of the

A Negative Control: Granulation tissue contains less collagen, fibroblasts, and blood capillaries and more inflammatory cells.

B Positive Control: Granulation tissue contains lower number of lymphocytes and macrophages. Collagen fibers are organized and the tissue is aligned.

C GA ointment (1%): Granulation tissue contains more collagen and fibroblasts with absence of inflammatory cells.

DISCUSSION AND CONCLUSION:

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of wound. It depends upon the repetitive abilities of tissue, type and extent of damage and general state of health of tissues (Jain et al., 2006). The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblasts, which start migrating into the wound gap along with the fibrin stands. The collagen composed of amino acid (hydroxyproline) is the major component of extracellular tissue, which gives strength and supports. Breakdown of collagen liberates free hydroxyproline could be used as an index for collagen turnover. Hence, in this study the model were used to access effect of grandiflorenic acid. The result of present study showed that grandiflorenic acid possesses a definite pro-healing action. In excision wound model ointment of leaves of grandiflorenic acid 1 % w/w topically showed better percentage wound closure effect against control and other treated groups on 15th day by enhanced epithelialization.

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