International Journal of Histology and Cytology Vol. 1 (5), pp. 047-049, May, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Histological assessment of *Plantago lanceolata* L. extract in accelerating wound healing

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Accepted 24 April, 2014

This study was conducted to evaluate the effects of topical application of *Plantago lanceolata* L. (PL; Plantaginaceae) extract on the rate of wound healing closure and histology of healed wound. An area of uniform wound 7 mm in diameter using circular punch was excised from the nape of the dorsal neck of all rats. The animal groups were topically treated with 0.75 and 1.5% of PL and two groups were treated as control and placebo groups. Macroscopically, wound dressed with PL extract significantly healed earlier than those treated with placebo and control groups. Histological analysis of healed wounds dressed with PL extract showed comparatively less scar width at wound site and healed wound contained less inflammatory cells and more collagen with angiogenesis compared to wounds dressed with placebo. Results of wound enclosure assessment showed that PL was significantly effective in wound enclosure. The best results (100.0% healing) were seen in third group (0.75% of PL extract) on 14th day. In conclusion, wounds dressed with PL extract significantly enhanced the acceleration of wound healing enclosure in rats.

Key words: *Plantago lanceolata*, wound healing, rat, histological study.

INTRODUCTION

Normal wound healing response begins the moment the tissue is injured. 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 begin to increase collagen production. Later, the epithelial tissue is regenerated (Souba and Wilmore, 1999). The objectives of the pharmacology of wound healing are to study the influence of various measures in wound management programmers on healing and to screen drugs that promote healing. Several materials have so far been used and are reported to affect healing differently.

However, intensive research in wound healing has not yielded, economic and efficacious pro-healing agent that could obviate the long hospitalization of patients following surgery and wound infliction (Shivananda et al., 2006). *Plantago lanceolata* L. (PL; Plantaginaceae), is a perennial plant species with a worldwide distribution and large ecological amplitude. Iridoid Glycosides (IGs) are a group of monoterpene-derived compounds that have been recorded in over 50 plant families (Bowers, 1991). The main IGs found in P. lanceolata are catalpol and its precursor aucubin (Jensen, 1991).

Several therapeutic effects including: Therapeutic effects on gastrointestinal, blood and respiratory (asthma and dyspnea) disorders have been described for the Plantago lanceolata in Iranian ancient medical books (Zargary, 1990). PL is used internally to suppress coughs associated bronchitis and upper respiratory inflamma tion, to reduce skin inflammation, treatment of wounds and as a laxative (Baytop, 1999).

In this study, we investigated wound healing activity of PL under pathological review which was based on anti-

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inflammatory characteristic of PL.

MATERIALS AND METHODS

Plant material preparation of the extract

Dried PL flowers were collected and Cold aqueous extract was prepared. Dried flowers were steeped for 6 h at 4°C in 300 ml distilled water, with constant stirring. The material was centrifuged and the supernatant was filter-sterilized and then freeze-dried.

Acute toxicity studies

The acute toxic study was used to determine a safe dose for PL. Thirty healthy Wistar rats (15 males and 15 females) were obtained from the Experimental Animal House, Faculty of veterinary, University of Urmia, and were divided equally into 3 groups labeled as placebo, 5 and 10% of PL. respectively. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms.

Mortality, if any, was observed over a period of two weeks. The animals were sacrificed on the 15th day. Hematological, serum biochemical and histological (liver and kidney) parameters were determined following standard methods (Bergmeyer, 1980; Tietz et al., 1983). Throughout the experiments, all animals received human care according to the criteria outlined in the "Guide for the Care and Use of laboratory Animals".

Experimental animals

32 Male Wister rats (190 to 210 g) of 10 weeks were obtained from the Experimental Animal House, Faculty of Veterinary University of Urmia and the rats were divided randomly into 4 groups of 8 rats each. The animals were housed separately (one rat per cage). The animals were maintained on standard pellet diet and tap water. Animal houses were in standard environmental conditions of temperature (22 ± 3°C), humidity (60 ± 5%), and a 12 h light/dark cycle.

Experimental induced excision wounds

After anesthesia induction with Xylizaine 2% and ketamine 10% (I.M. 60 mg/kg), rats were fixed in ventral posture on surgery table. Then the dorsal area from scapula to ilium were scrubbed and prepared to surgery. Two circle-shapes, full thickness surgical wounds with 7 mm diameters in both side of the backbone, 1 cm away from backbone and 5 cm away from each other were made with biopsy punch. With this excisional wounding method, epidermis, dermis, hypodermis and Panniculus Carnosus layers were removed completely (Luisa and DiPietro, 2003). Wound contraction was monitored by measuring wound area, on alternate days till the wounds were completely healed. Wound contraction was calculated as percentage reduction in wound area.

Topical wound application

After being made of surgical wound, all rats randomly was colored with none toxic color and divided to three groups. In group A, 0.75% PL extract was administered. Group B received 1.5% PL. group D as placebo were administrated with Eucerin and Vaseline and group C as control group did not received any administration. All extracts were applied in topical route. All rats were followed 21 days later. Daily observation was performed and any wound fluid or any evidence of infection or other abnormalities were noted.

Histopathological evaluation of healed wounds

The skin samples were obtained during days 3, 7, 14 and 21, from all groups of animals and were processed for histological study. The samples fixed in formalin and installed on slides, stained with Hematoxylin and Eosin and were reviewed under light microscope. Recorded factors were scar, inflammatory cells, kind of inflammatory cells, angiogenesis, fibroplasia, epithelial growth, hyperemia, collagen density and Fibroblastic aggregation.

Statistical analysis

All values are reported as mean ± S.D, the statistical differences among groups were assessed using Duncan multiple range test and analysis of variance (ANOVA). A value of $p<0.05$ was considered significant. Statistical analysis was performed using SAS 9.1 for Windows.

RESULTS

Acute toxicity

Acute toxicity of *P. lanceolata* was carried out on animal at dose of 5 and 10% and animals were kept under observation for 14 days. All animals remained alive and did not show any significant differences between control and treated groups. We concluded that *P. lanceolata* orally administrated to rats was safe and no side effect was seen even at highest doses.

Wound healing activity

Groups: A: 75%, B: 1.5%, C: Control, D: Placebo

On 3rd day angiogenesis in A group was more than B group. But it was same between B and C groups. On 7th day, both hyperemia and bleeding were same in all groups. Leukocytes in A group were less than B group but it was significantly high in C group compare to A and B. There was no remarkable difference in angiogenesis between groups. Collagenation was not noticeable. The main difference between A group and other groups on 7th day was epithelization which was macroscopically higher than other groups. Scar formation in treated group was also better than C or D.

During 14th day, hyperemia was equal both in A and C but it was high in B. There was no bleeding in A but both B and C showed a bit of bleeding. There was no sign of inflammatory in A and B but leukocytes were seen in C group. Angiogenesis was still observable in A and B. Epithelization was remarkably up in A and B.

On 21st day there was no sign of hyperemia in C but it was seen in A and B. There was no inflammatory cell in A and B but in C group lymphocytes were still in the site

Table 1. Effect of B. *Plantago lanceolata* extract on percentage (%) wound healing in experimental rats.

No.	Group	3 days	7 days	14 days	21 days
1	Control	26.04±0.49	45.25^{c}±1.35	71.54^{d}±0.74	95.13^{b}±1.38
2	Placebo	26.14±1.42	46.42^{c}±0.22	75.35^{c}±0.81	100^{a}±0.0
3	0.75%	26.30±0.37	71.47^{b}±1.17	100^{a}±0.00	100^{a}±0.0
4	1.5%	26.44 ±1.66	75.29^{a}±1.20	85^{b}±0.73	100^{a}±0.0
Significance		NS	*	**	**

* All expressed as mean and standard deviation (S.D). Mean in columns with different letters were significantly different (NS Not Significant *p<0.05, **p<0.01).

and there was no difference between groups according to epithelization and scar formation.

Wound enclosure

Percentages of wound healing have been documented in Table 1. 3 days after drug application no statistical significant differences were seen between groups (NS), although treated groups showed better wound enclosure percentage. Topical ointment demonstrated its effectiveness and treated groups had significant difference compare to control and placebo groups in 7th day. In 14th day, complete enclosure was seen in A group and B group showed higher closure compare with placebo and control groups. Consider the fact that during whole study placebo showed a slight effect on better healing. As it can be seen, 21^{st} day of study all groups showed complete closure except control group which was not completely closed in healing site.

DISCUSSION

It is important to note that throughout the period of wound treatment, the PL extract did not cause irritation or pain to the animals as the rats neither show any signs of restlessness nor scratching/biting of wound site when the extract were applied. All the surgical interventions were carried out under sterile conditions and animals were closely observed for any infection. This is very important that the control microbial infection is necessary for better healing and its management (Muhammad and Muhammad, 2005).

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 contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction resulting in a smaller amount of apparent scar tissue (Midwood et al., 2004). In the present study, topical application of PL extract significantly accelerated the rate of wound healing, and histology, healed wound contain comparatively less inflammatory, more collagen and angiogenesis. Topical application of PL extracts demonstrated its effectiveness and treated groups presented significant difference compare to control and placebo groups in 7th and 14th days however, group A showed complete wound closure (100%) sooner (14th) than other groups (groups B, C and D). Wound healing effects may be due to regulation of collagen expression (Bonte et al., 1993).

Similarly, enhanced healing activity has been attributed to collagen formation and angiogenesis (Shukla et al., 1999; Trabucchi et al., 1986). Collagen played a central role in the healing of wounds and it is a principal component of connective tissue and provides a structural framework for the regenerating tissue (Cohen et al., 1992); although we did not completely measure collagen rate in wound site, wound enclosure results show remarkable collagenation in treated groups (Table 1). Angiogenesis in granulation tissues improves circulation to the wound site thus providing oxygen and nutrients essential for the healing process (Szabo et al., 1995) that includes re-epithelization (Habibipour et al., 2003) and showed that histological analysis of the treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis, and neovascularization, which resulted in an increased wound tensile strength and accelerated healing of wound. It is proved that PL has an anti-inflammatory activity (Herold et al., 2003; Wegener and Kraft, 1999) which might be a reason in accelerating wound healing.

According to this characteristic of PL, inflammatory cells rate will decrease in the wound site which lead to better wound healing. Extract from PL and Plantago major are antiphlogistic in carrageenan and PGE1-induced inflammations in rats (Shipochliev, 1981). Aceteoside, the main phenylethanoid from PL inhibits arachidonic acid induced mouse ear oedema (Murai et al., 1995). Therefor PL can inhibit oedema in wound site as it did in present study some wound healing process could be accelerated under none-oedema condition in wound site.

Compounds such as the iridoid glycosides aucubin and catalpol, the aglycone aucubigenin, and caffeic acid derivatives including plantamajoside and acteoside have been isolated from *Plantago* spp. and have demonstrated antimicrobial activity (Samuelsen, 2000; Blumenthal et al., 2000) thus PL can decrease microbe volume in wound site which is a useful trend to accelerate wound healing.

In this study, we demonstrated that extract from PL was beneficial in wound healing base on its anti-microbial and anti-inflammatory which helped to faster wound contraction and better histological properties of treated wounds.

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