Original Article

Antidiabetic and Wound Healing Activity of *Catharanthus roseus* L. in Streptozotocin-Induced Diabetic Mice

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ABSTRACT

Ethnopharmacological Relevance: Natural antioxidants due to their radical scavenging and lipid peroxidation ability are considered as possible protection against delayed wound healing in diabetic condition.
The Aim of the Study: The objective of our study was to evaluate the antidiabetic and wound healing activity of the leaf extract of *Catharanthus roseus* L in mice.
Materials and Methods: The methanolic extract of leaf of *Catharanthus roseus* L at a dose of 200 mg/kg and 400 mg/kg body weight was induced through intraperitonial in mice. To create wounds, a single full thickness 1.0 cm diameter superficial excision was made on the mid-dorsum of each mouse. The measurement of the wound diameter was taken on 1, 3, 7 and 10 days by using transparency paper and permanent marker.
Results: The plant drugs increased the rate of wound contraction and significantly decrease the glucose level in blood. The results were compared with control group of mice that were kept on only saline water. The wound closure was optimal at the dose of 200 mg/kg body weight. The extract of *Catharanthus roseus* L significantly increased the wound contraction and formation of collagen fibres in the mice compared with controls ($P < 0.001$).
Conclusion: The methanolic extract of *Catharanthus roseus* L significantly increases the wound contraction in mice that promises to overcome the delayed wound healing in diabetic condition.

Keywords: Phenolic Compounds, Antidiabetic, Antioxident, Wound Healing.

INTRODUCTION

India possesses a rich biodiversity of the medicinal plants that were still not explored completely. The need for the novel pharmaceutical products out from the plant has attained a great interest in the present biomedical researches due to potent source of natural antioxidant[1] *Catharanthus roseus* L is a medicinal plant that shows different type of pharmacological activities like antimicrobial, antioxidant, antidiarrheal and antidiabetic[2]. The aim of this study was to evaluate the wound healing activity of extracts from the leaves in normal and Streptozotocin induced diabetic mice. *Catharanthus roseus* L was found to possess a large number of phytochemicals. Alkaloids are found in higher percentage due to that it secure wound healing and antidiabetic properties. Especially two major alkaloids in *Catharanthus roseus* L such as vinblastine and vincristine was developed for anticancer drugs[3].

This study explores the wound healing activity of *Catharanthus roseus* L with their recent advancement in treating chronic wounds in diabetic condition. Diabetes mellitus is one of the major contributors to chronic wound healing problems[4]. When diabetic patients develop an ulcer, they are exposed to high risk for major complications including infection and amputation. It has been suggested that diabetes impairs wound healing through disruption of local cytokine production, notably platelet derived growth factor (PDGF), tumor necrosis factor α (TNFα), interleukin -1β, and vascular endothelial growth factor (VEGF), reduced biosynthesis or accelerated degradation of newly synthesized collagen[5,6]. Wound healing process involves several steps, including contraction, granulation, epithelization and formation of collagen[7]. This is mainly achieved by synthesis of new connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the major component that ultimately contributes to wound strength. Factors such as age, obesity, malnutrition, and macrovascular disease may contribute to wound infection and delayed wound healing in the type II diabetic patient. In addition, hyperglycemia caused by decreased insulin availability and increased resistance to insulin can affect the cellular response to tissue injury. Studies of the immune cells necessary for wound healing, such as PMN leukocytes and fibroblasts, as well as studies of injured tissue suggest that there is a delayed response to injury and impaired functioning of immune cells in diabetes mellitus.

Tannins promotes the wound healing through several cellular mechanisms, chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts. Phenolic compounds like phenolic acids, flavonoids and tannins are important plant metabolites that play a significant role in the diabetic wound healing. Tannins act as free radical scavengers, tri-terpenoids and flavonoids that promote wound healing due to their astringent and antimicrobial property and saponins due to their antioxidant and antimicrobial activity which appear to be responsible for wound contraction and elevated rate of re-epithelialization. Flavonoids also posses potent antioxidant and free radical-scavenging effect, enhancing the level of antioxidant enzymes in granuloma tissue.

MATERIALS AND METHODS

Collection and identification of plant material

The leaf of *Catharanthus roseus* L were collected from the Faculty of

Ayurveda, Banaras Hindu University, Varanasi. The plant was identified and authenticated by the Department of Botany, Banaras Hindu University, Varanasi. The material was shade dried, pulverized and preserved in air tight containers.

Extraction of plant material

Methanolic extraction

The methanolic extraction of dried powder (500 gm) of the leaves was prepared by using Soxhlet apparatus at 65^0C. The extracted materials was then kept in water bath to evaporate solvent totally and then kept on a rotary shaker at 190-220 rpm for 6 h to make the final volume one fourth of the original volume and stored at 4^0C in airtight bottles. The yield of the extract was 5.6 %. The methanolic extract was then subjected to phytochemical analysis and wound healing activity of diabetic mice.

Phytochemical screening methods

The extracts were tested for alkaloids using Mayer's, Wagner's, Bouchardt's, and Drangendorf's reagents, according to the method described by Maldoni[8]. Glycosides were determined using Benedict reagents[9] and saponins were assessed by foam test. The polyphenolic compounds were tested with ferric chloride solution with hydrochloric acid and for flavonoids using 1% aluminium chloride solution in methanol, concentrated hydrochloric acid, magnesium turning, and potassium hydroxide solution.

Test for saponins

Boiled 300 mg of extract with 5 ml water for two minutes. Mixture was cooled and mixed vigorously and left it for three minutes. The formation frothing indicates the presence of saponins.

Test for tannins

To an aliquot of the extract added sodium chloride to make to 2% strength. Filtered and mixed with 1% gelatin solution. Precipitation indicates the presence of tannins.

Test for Triterpenes

300 mg of extract mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicates the presence of triterpenes.

Test for alkaloids

300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink colour which indicates the presence of alkaloids.

Test for flavonoids

The presence of flavonoids was determined using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnins, and potassium hydroxide solution.

Animals

All experiments were performed on 7 to 8 week old male swiss albino mice with an average weight of 25±1 g. The animals were individually kept under laboratory conditions at the Department of Biochemistry, Banaras Hindu University, Varanasi. The mice were divided into two groups diabetic (D) and non-diabetic (ND) comprising five animals in each groups. The first five groups were considered as diabetic and assigned as DC, DM, DM+E_1, D+E_1 and D+E_2 where M for metformin and E_1 and E_2 respectively represent the different concentration of plant extracts. Diabetes was induced by giving intraperitonial

streptozotocin injection for five consecutive days. The animals were confirmed for diabetes before the start of experiment. The serum glucose level was measured by glucose oxidase-peroxidase method using glucose test kit (Span diagnostics Ltd., India). The other five groups of mice were considered as non-diabetic and assigned as NDC, NDM, NDM+E_1, ND+E_1 and ND+E_2.

Wound creation

To develop wounds, a single full thickness 1.0 cm diameter superficial excision was made on the mid-dorsum of each diabetic and non-diabetic mouse at day 0. The measurement of the wound diameter was taken on 1st, 7thand 13th days by using transparency paper and permanent marker.

Extract administration

The DC and NDC (Control), DM and NDM (60 mg/kg body weight in 200 µl ddH_2O), DM+E_1 and NDM+E_1 (30 mg/kg body weight Metformin in 100 µl ddH_2O + 100 mg/kg body weight extract in 100 µl ddH_2O), D+E_1 and ND+E_1 (200 mg/kg body weight extract in 200µl ddH_2O), D+E_2 and ND+E_2 (400 mg/kg body weight extract in 200µl ddH_2O) were injected for 14 consecutive days starting from day zero through intraperitoneal tube. The level of glucose were measured on 1st, 7th and 13th post wounding days of both diabetic and non-diabetic mice.

Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) using SNK test (Students-Newmann-Keuls) with sigma stat 3.5. The *p*-value less then 0.05 were considered to be significant (level of significance*=0.05, **=0.01, ***=0.001). Data were represented as mean ± SD. All the studies were performed in quadruplicate.

RESULTS

Phytochemical analysis

The phytochemical analysis of *Catharanthus roseus* L revealed the presence of phenols, tannins, alkaloids, saponin and flavonoids. The presences of these phytochemicals are considered to be responsible for wound healing activity in diabetes (Table 1).

Anti-diabetic plant extract decreases serum glucose level in diabetic mice

Administration of plant extract resulted in a significant decrease ($P<0.001$) in serum glucose level on day 7 and 13 in case of diabetic mice as compared to the diabetic controls. A less significant decrease ($P<0.05$) was observed in case of mice treated with metformin on day 7 but on day 13 this decrease was highly significant ($P<0.001$) mice (Table 2).

Calculation of wound contraction

The measurement of the wound areas were taken on the day 1st, 7th and 13th using transparency paper and a permanent marker. The wound areas were recorded and measured on graph paper. The plant extract increased the rate of wound healing in the diabetic mice (Figure 1). The wound closure was optimal in the diabetic group of D+E2. The results are summarized in Table-3.

DISCUSSION

Wound healing occupies an important and developing field of research in modern biomedical sciences. Plants and their extracts have immense potential for the management and treatment of wounds. The phyto-medicines for wound healing are not only cheap and affordable but are also purportedly safe as hyper sensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing

and regeneration of the lost tissue by multiple mechanisms.

Wound healing consists of orderly progression of a series of events that establish the integrity of the damaged tissue. Normal wound healing can be adversely affected by many factors. If the healing fails to progress in the usual stepwise manner then it may lead to development of chronic wound. Chronic wounds afflict a very large number of patients and seriously reduce their quality of life[10]. Treatments that exist today, however, are often expensive and exhibit major adverse effects. Scientists who are trying to develop newer drugs for wound care are looking toward the natural sources because of their lesser side effects.

The methanolic extract of *Catharanthus roseus* L significantly speed up the healing process and provide the strength to collagen tissue. The preliminary phytochemical analysis of the leaf extract showed the presence of tannins, tri-terpenoids and alkaloids[11,12]. Any one of the observed phytochemical constituents present in Leaf of *Catharanthus roseus* L may be responsible for the wound healing activity. Recent studies have shown that phytochemical constituents like flavanoids and tri-terpenoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties[13,14], which appear to be responsible for wound contraction and increased rate of epithelialisation[15]. Further phytochemical studies are in progress to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity[16].

The results of this study can be justified by the facts that the methanolic extract of leaf of *Catharanthus roseus* L enhances the faster lay down of collagen fibres and improves the antioxidant status in the wound of diabetic animals.

CONCLUSIONS

The present study has demonstrated that a methanol extract of *Catharanthus roseus* leaves has properties that render it capable of promoting accelerated wound healing and antidiabetic activity when compared with normal controls. Wound contraction, increased tensile strength, increased hydroxyproline content and antimicrobial activity support further evaluation of *Catharanthus roseus* in the topical treatment and management of wounds and diabetic.

REFERENCES

1. Dimitrios B, Sources of natural phenolic antioxidants. *Trends Food Sci. Technol* 2006; 17: 505-512.
2. A. Akhtar, M. Rashid, M. I. I. Wahed *et al*., "Comparison of long-term anti-hyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) And *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats," *Research Journal of Medical Sciences*, vol. 2, pp. 29–34, 2007.
3. El-Sayed A, Cordell GA: Catharanthamine, a new antitumor bisindole alkaloid from *Catharanthus roseus*. *J Nat Prod* 1981, 44(3):289-293.
4. Goodson W.H and T.K. Hunt., Wound healing and the diabetic patients. *Surg. Gynecol. Obstet* 2010; 149: 600-608.
5. Rosenberg L, de la Torre J, Wound Healing Growth Factors. *Emedicine. Com. Accessed* 2008; 20.
6. Bailey J.B, The fate of Collagen implants in tissue defects wound repair. *Repair Regen* 2000; 8: 5-12.
7. Ruszczak Z, Effext of Collagen matrices on Dermal wound healing. *Advance Extract Delivery Reviews* 2003; 55(12): 1595-1611.
8. Maldoni, "Alkaloids: isolation and purification," *Journal of Chemical Education*, vol. 68, no. 8, pp. 700–703, 1991.
9. X. A. Dom´ınguez, *M´etodos De Investigaci´on Fitoqu´imica*, Limusa, M´exico. Mexico, 1st edition, 1973.

10. S. Guo and L.A. Dipietro. Factors Affecting Wound Healing. Critical Reviews in Oral Biology & Medicine. *J Dent Res* 2010; 89 (3): 219-229.
11. F. Ferreres, D. M. Pereira, P. Valent˜ao, P. B. Andrade, R. M. Seabra, and M. Sottomayor, "New phenolic compounds and antioxidant potential of *Catharanthus roseus*," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, pp. 9967–9974, 2008.
12. Strodtbeck, F, Physiology of wound healing. *Newborn Infant Nurs. Rev* 2001; 1: 43-52.
13. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tnanka T, linuma M: Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol* 1996, 50:27-34.
14. Scortichini M, Pia Rossi M: Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill). *J Appl Bacteriol* 1991, 71:109-112.
15. Shivananda Nayak B: *Cecropia peltata L* (*Cecropiaceae*) Has Wound Healing potential-A preclinical study in Sprague Dawley Rat model. *International Journal of Lower Extremity Wounds* 2006, 5:20-26.
16. Nayak BS, Vinutha B, Geetha B, Sudha B: Experimental evaluation of Pentas *lanceolata* for Wound healing activity in rats. *Fitotherapia* 2006, 76:671-675.

Table 1. Qualitative analysis of leaf of *catharanthus roseus* L

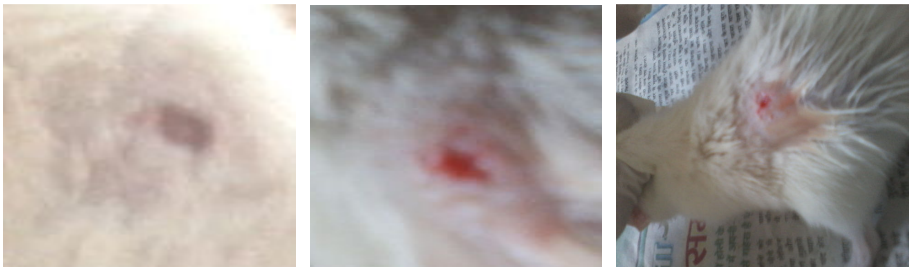
Plant species	Alkaloids (%)	Phenols (%)	Tannin (%)	Flavonoids (%)	Saponin (%)
Leaf of Catharanthus roseus L	+	+	+	+	+

Table 2. Level of serum glucose in various diabetic and non-diabetic groups of mice

Groups	Level of serum glucose (mg/dl)		
	1st Day	7th Day	13th Day
NDC	103±3.10	98±3.14	93±3.45
NDM	108±3.13	104±4.26	89±3.65
NDM+E_1	107±3.82	102±3.46	96±4.32
ND+E_1	111±3.58	99±3.37	94±3.23
ND+E_2	106±3.12	94±3.18	88±3.41
DC	222±4.86	236±3.97	264±3.61
DM	198±3.24	155±3.79	142±4.11
DM+E_1	211±3.33	142±3.34	118±3.41
D+E_1	219±3.36	138±3.98	112±3.78
D+E2	216±2.87	132±3.54	108±4.21

Table 3. Wound diameter in various diabetic and non-diabetic groups of mice

Groups	Wound contraction (mm^2)		
	1st Day	7th Day	13th Day
NDC	100±2.92	38.96±1.98	3.28± 0.98
NDM	100±2.80	32.17±1.89	0.37±0.87
NDM+E_1	100±2.45	39.75±1.84	2.11±0.97
ND+E_1	100±2.67	48.21±1.99	0.76±0.78
ND+E_2	100±2.89	51.45±2.15	0.49±0.89
DC	100±2.81	81.32±1.96	59.34±1.95
DM	100±2.79	61.26±2.18	22.72±1.10
DM+E_1	100±2.65	83.61±2.26	24.32±1.06
D+E_1	100±2.98	66.45±2.07	15.23±0.99
D+E_2	100±2.96	62.87±2.21	9.12±1.02



(1) (2) (3)

Figure 1. Photograph showing various stages of wound healing activity in different group of diabetic mice respectively 1 (DM), 2 (D+E1), 3 (D+E2)