Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 5 (3): 328-334, 2010
ISSN 1816-4951 / DOI: 10.3923/jps.2006.247.253

Wound Healing Activity of *Desmodium gangeticum* in Different Wound Models*

[1]V. Jain, [2]V. Prasad and [1]R.S. Pandey
[1]RKDF college of Pharmacy, Bhopal, India
[2]Department of Pharmaceutical Science,
Nagpur University Campus, Nagpur, India

Abstract: *Desmodium gangeticum* (DG) aqueous extract was investigated for its wound healing potential on different experimental models of wounds in rats. The aqueous extract of aerial part of DG, in powdered form was incorporated in ointment (10% w/w dried powder in simple ointment base) and was evaluated for wound-healing potential in an excision, incision and dead space wound model in rats. The DG ointment showed significant responses in all three-wound types tested when compared with the control group. The effect produced by the DG ointment, in terms of wound contracting ability, wound closure time, tensile strength of the wound, regeneration of tissues at wound site were comparable to those of a standard drug povidone iodine ointment.

Key words: Desmodium, wound healing, ointment, excision wound, incision wound, dead space wound

Introduction

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation.The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collegenation and tissue remodeling (Fulzele *et al*., 2002). In India, there has been interest in the potential of medicinal plant to for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda (Biswas and Mukherjee, 2003).

Desmodium gangeticum (L.) DG. (Family Leguminaceae) is a small shrub of tropical region which has been used in Indian system of medicine as a bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic, in inflammatory conditions of chest and various other inflammatory conditions due to 'vata'disorders (Kirtikar and Basu, 1987). The aqueous extract of this species has been reported to show severe antiwrithing activity, moderate central nervous system (CNS) depressant activity (Jabbar *et al*., 2001) and antileishmanial activity (Singh *et al*., 2005). Gangetin, a pterocarpnoid from DG. has been shown to possess anti-inflammatory and analgesic activities (Rao *et al*., 2004). Total alkaloids of this species showed anticholinesterase, smooth muscle stimulant, CNS stimulant and depressant responses (Ghosal and Bhattacharya, 1972). It also known to possesses antioxidant activity (Govindarajan *et al*., 2003). Chemical studies on the DG revealed the presence of alkaloids, pterocarpnoid, flavnoid and isoflavanoid glycosides (Avasthi and Tewari, 1955; Mishra *et al*., 2005).

Corresponding Author: V. Prasad, Department of Pharmaceutical Science, Nagpur University Campus, Nagpur, India Tel: +91-522-2212411-18/-4302 Fax: +91-522-2223938, 2223405

Originally Published in Journal of Plant Sciences, 2006

DG is used as Ayurvedic drug in India from centuries; however, there was no scientific report available on traditional claims of the water decoction of aerial portion of this plant. Tribal people in India used aerial part of this plant for treatment of open wounds. Further, chemical constituents present in aqueous extract shows presence of flavnoids and terpenes, which are known to promote wound healing (Manjunatha *et al*., 2005). Therefore, present study aims to assess the wound healing activity of aqueous extract of *D. gangeticum* in different wounds model like excision, incision and granuloma. As the use of single wound healing model is inadequate, as it doesn't represent all the phases and wound types. Hence, three different models are selected to assess the activity and further histopathological studies will helps to assess the effect of DG on various phases of wound healing which can occur simultaneously but are independent of each other.

Materials and Methods

Plant Material Plant of DG was collected from Chitrakoot, Madhya Pradesh (India) during the month of October. Botanist of Barkatullah University, Bhopal authenticated the plant. The study was carried out in RKDF College of pharmacy, Bhopal in month of July.

Preparation of Plant Extract

The dried aerial parts were powdered and passed through 10 mesh sieve. The coarsely powdered materials (500 g) were soaked in distilled water in the ratio of 1:20 (w/v). The extracts were filtered, pooled and first concentrated on rotavapour and then dried in freeze dry system/Freezone® 4.5 with high vacuum, at -40°C (Dried extract yield was 18.1% (w/w) and 21.7% from root and aerial part of DG, respectively). For the pharmacological tests, the dried extract of root and aerial parts of DG was powdered and incorporated in simple ointment base 10% (w/w) and were used throughout our experimental studies.

Wound Healing Activity

Animals

Wistar rats of male sex weighing 150-200 g were selected for assessing the wound healing activity in excision, incision and dead space wound models. Animals were divided in to three groups, reference, control and treatment with six animals in each group. Animals were housed individually with free access to food and water, the basal food intake and body weights to nearest gram were noted. The animals were starved for 12 h and anaesthized with anaesthic ether using open mask method prior to wounding.

Excision Wound Model

The rats were depilated at the dorsal side and a circular piece (300 sq. mm) in area with full thickness skin was excised from dorsal inter-scapular region (Morton and Melone, 1996) The day on which wound was made was consider as day 0.

The 0.2% Povidone, simple ointment base and DG incorporated ointment to reference, control and treatment group was applied every day till the wound was completely healed.

The animals were monitored for the wound contraction and period of epithelization.The percent wound closure was recorded on day 4th, 8th, 12th, 14th and wound area was traced and measured planimetrically. The actual value was converted in to percent value taking size of wound at the time of wounding as 100%. The number of days required for falling scar without any residual raw wound gave period of epithelization.

Incision Wound Model

To 5 cm long Para-vertebral incisions were made through the entire thickness of skin at a distance of about 1.5 cm from the midline on each side of depilated back of the rat. After mopping the wound dry, intermittent sutures were placed 1cm apart, using surgical nylon thread and a curved needle (No.11). The 0.2% Povidone, simple ointment base and DG incorporated ointment to reference, control and treatment group was applied topically daily for 10 consecutive days. On the 7th day sutures were removed and on 10th day rats were sacrificed by exposing them to a higher dose of anesthetic ether and tensile strength were measured by adopting continuous constant water flow technique (Lee, 1968).

Dead Space Wound (Granuloma Studies)

The Physical changes in the granuloma tissue were studied in this model. The subcutaneous dead space wounds were inflicted in the region of axilla and groin by making a pouch through a small nick in the skin. The Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced in to the pouch. Each animal received 2-grass piths in different locations. Implantation of grass pits induced granuloma formation (Taranalli and Juppest, 1996).

The wounds were sutured and mopped with an alcoholic swab. Animals were placed into three individual cages after recovery from anesthesia. The 0.2% Povidone, simple ointment base and DG incorporated ointment to reference, control and treatment group was applied topically daily for 10 consecutive days. Excision of the granulomas from the surrounding tissue was performed on the 10th post-wound day under light ether anesthesia. Granulomas surrounding the grass piths were excised and slit open. The tensile strength of tissue piece (obtained by trimming the rectangular strip of granular tissue) measuring about 15 mm in length and 8mm width was determined on 10th post wounding day by adopting continuous water flow technique (Yeo *et al*., 2000). The granulation tissue removed on day 10 was also analyzed for hydroxyl proline content. The hydroxyl proline content was assayed spectrophotometrically using the technique described by Woessner (1961) at 577 nm.

Histopathological Studies

The sections from regenerated tissue (10 days) were studied under the light microscope for keratinization, epithelization, fibrosis, inflammation and non-vascularization. The results studied were numbered from 1 to 5 with 5 standing for maximum similarity and 1 standing for least similarity from normal tissue in wounded area in the control and treatment group (Rao *et al*., 1996).

Statistical Analysis

Results obtained from wound models have been expressed as mean± SEM and were compared with the corresponding control (simple ointment) values. P-values were calculated by ANOVA followed by Dunnets test. The percentage of Wound Contraction was calculated, as a percentage of the corresponding 0-day's (original) wound area.

Results

Excision Wound Study

The progress of the wound healing induced by powder ointments (10% w/w) treated groups, simple ointment (control) treated group and Povidone (standard drug) treated group of animals are shown in Table 1. It is observed that the wound contracting ability of the DG ointment was significantly greater t han that of the control (i.e., simple ointment treated group). The 10% (w/w)

Table 1: Effect of DC ointment on excision wounds

Groups	Wound contraction (%) Day4	Day8	Day10	Day12	Day14	Period of Epithelization
Simple ointment (Control)	19.30±1.08	39.24±3.03	49.81±3.02	60.38±1.93	72.26±1.53	32.34±1.27
DC ointment 10% w/w (Treatment)	53.25±1.04*	79.30±2.02*	94.01±1.32*	99.18±1.36*	99.15±1.62*	17.24±1.23*
Povidone Iodine Ointment (Standard)	51.93±2.12*	81.77±1.02*	96.85±2.66*	98.90±1.64*	99.12±1.07*	16.02±1.05*

All the results are reported as mean±SD of each group of rat. One way ANOVA followed by dunnets test * $p<0.001$ as compared to control group

Table 2: Effect of DC ointment on incision wound and granulomal (dead space wound)

Groups	Incision wound model Tensile strength (g)	Dead space model Granuloma weight (g/100g)	Breaking Strength (g)	Proline content
simple ointment (control)	240.43±8.21	0.029±0.0045	211.33±4.88	634.16±9.55
10%DC ointment (Treatment)	443.97±8.02*	0.078±0.0036*	423.66±5.92*	841.46±18.54*
Povidone ointment (Standard)	435.65±5.16*	0.079±0.0073*	417.36±6.60*	825.34±15.39*

All the results are reported as mean±SD of each group of rat. One way ANOVA followed by dunnets test, * $p<0.001$ as compared to control group

Table 3: Histological examination of granuloma wounds treated with DC ointment at the end of 10 days

Parameter	Simple ointment (control)	DC ointment (treated)	Povidone (Standard)
Keratinization	1.1±0.11	3.9±0.55*	4.2±0.50*
Epithelization	1.3±0.31	4.0±0.33*	4.0±0.29*
Fibrosis	2.1±0.46	4.2±0.71*	4.1±0.67*
Collagenation	2.3±0.51	4.1±0.38*	4.3±0.52*
Neovascularization	1.6±0.25	4.2±0.45*	4.5±0.87*

All the results are reported as mean ± SD of each group of rat. One way ANOVA followed by dunnets test, * $p<0.05$ as compared to control group

DG ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e., Povidone ointment treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 10% w/w extract ointment treated group (15 days for 100% contraction which was almost similar to that of the Povidone treated group). The epithelization period was found to be decreased in DG ointment treated group, which was comparable with Povidone treated group.

Incision Wound Model

The tensile strength of the 10% w/w DG ointment treated group and the povidone ointment treated group was comparable to each other. Thus both DG ointment and the standard povidone ointment showed a significant increase in tensile strength in the 10 days old wound (Table 2).

Dead Space Wound Model

The measurement of the effect of the fruit powder ointment and standard drug on the Granuloma weight and it breaking strength along with hydroxyl proline content in 10 day dead space wound is shown in Table 2. DG ointment treated group's shows significant increase in granuloma weight and

its breaking strength, which was compare to the standard treated group. DG ointment treated group and the standard DG treated group also showed a significant increase in hydroxyl proline content.

Histopathological Studies

The various parameters studied in histopathological examination of the tissues of the wound area treated with the DG ointments (10%w/w), 0.2% w/w Povidone ointment and simple ointment to treated groups are depicted in Table 3. Treatment was found to promote keratinization, epithelization, fibrosis, collegenation and neovascularization and values were compared to standard treatment in 10-day-old wound.

Discussion

The aim of this article was to study wound-healing potential of aqueous extract of *Desmodium gangeticum* (DG). This plants indigenous to India and is mentioned in the Ayurveda for wound healing and other pharmacological activities. (The Wealth of India, 1996). The need for the study arose from the fact that Wound-healing process involve various phases viz., granulation, collegenation, collagen maturation and sear maturation and very few drugs are available that can act directly on the all the phases of wound healing process. Drugs, which influence one phase, may not necessary influence other (Duke *et al*., 2002).

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 the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue. In the present investigation, DG ointment promotes wound contraction and increased rate of epithelialisation in excision wound which may be due to presence of phytochemical constituents like flavonoids, tannins. These constituents are known to promote wound contraction and increased rate of epithelialisation due to their astringent and antimicrobial property (Getie *et al*., 2002).

In incision wound DG ointment resulted in increased mean tensile strength that reflects better collagen synthesis (Mukerjee *et al*., 2000). Tensile strength depends on the Vander Walls interaction among the hydrogen ion bonds of the triple helix collagen leading to twisting of the collagen fibers. The more twisting, the greater the tensile strength, hence better the healing of wounds (Mian *et al*., 1992).

The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen is the major component of extra cellular tissue, which gives support and strength and is composed of amino acid hydroxyproline (Gupta and Gupta, 1985). The data depicted in Table 2 reveal that the hydroxyproline content of the granulation tissue of the animals treated with DC ointment was significantly increased when compared to the control group, indicating increased collagen turnover. Increase in granuloma dry weight and its breaking strength, which is indicative of higher protein content and collagen maturation respectively (Chitra *et al*., 1998). Histopathogical studies in granuloma tissue further indicate strengthen in tissue integrity by DG ointment treatment by enhancing Keratinization, epithelization, fibrosis, collagenation and angiogenesis.

The above observations suggest that DG ointment promotes wound healing through mainly by collagen formation and enhancing tissue integrity. Further phytochemical studies are in progress where the aqueous extract will be subjected to further fractionation and purification to identify and to isolate

the active compound(s) responsible for these pharmacological activities. The present findings provide scientific evidence that aq extract of aerial part of *Desmodium gangeticum* as potential wound healer.

References

Avasthi, B.K. and J.D. Tewari, 1955. A preliminary phytochemical investigation of *Desmodium gangeticum* DC. (Leguminosae). J. Am. Pharm. Assoc. Am. Pharm. Assoc. (Baltim)., 44: 625-627.

Biswas, T.K. and B. Mukherjee, 2003. Plant medicine of Indian origin for wound healing activity: A review. Ind. J. Lower Ext. Wounds, 2: 123-132.

Chitra, P., G.B. Sajithlal and G. Chandrasekharan, 1998. Influence of aloe vera on collagen turnover in healing of dermal wounds in rats. Ind. J. Exp. Biol., 36: 896-901.

Duke, J.A., M.J. Bogenschutz-Godwain, J. Ducellier and P.K. Duke, 2002. Handbook of Medicinal Herbs. London, CRC press.

Getie, M., G. Mariam, R. Reitz and R.H. Neubert, 2002. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae). Pharmazie, 57: 320-2.

Fulzele, S.V., P.M. Satturwar, S.B. Joshi and A.K. Dorle, 2002. Wound healing activity of hingvadya ghrita in rats. Indian Drugs, 39: 606-609.

Ghosal, S. and S.K. Bhattacharya, 1972. Desmodium alkaloids. II. Chemical and pharmacological evaluation of *D. gangeticum*. Planta Med., 22: 434-440.

Govindarajan, R., S. Rastogi, M. Vijayakumar, A. Shirwaikar, A.K. Rawat, S. Mehrotra and P. Pushpangadan, 2003. Studies on the antioxidant activities of *Desmodium gangeticum*. Biol. Pharm. Bull., 26: 1424-1427.

Jabbar, S., M.T. Khan and M.S. Choudhuri, 2001. The effects of aqueous extracts of *Desmodium gangeticum* DC. (Leguminosae) on the central nervous system. Pharmazie., 56: 506-508.

Kirtikar, K.R. and B.D. Basu, 1987. Indian Medicinal Plants. 2nd Edn., Delhi, pp: 757-759.

Lee, K.H., 1968. Studies on the mechanism of action of salicylate. II. Retardation of wound healing by aspirin. J. Pharm. Sci., 57: 1042-1043.

Manjunatha, B.K., S.M. Vidya, K.V. Rashmi, K.L. Mankani, H.J. Shilpa, S. Singh and D. Jagadeesh, 2005. Evaluation of wound-healing potency of *Vernonia arborea*. Ind. J. Pharmacol., 37: 223-226.

Mian, M., F. Beghe and E. Mian, 1992. Collagen as a pharmacological approach in wound healing. Intl. J. Tiss. React., 14: 11-14.

Mishra, P.K., N. Singh, G. Ahmad, A. Dube and R. Maurya, 2005. Glycolipids and other constituents from *Desmodium gangeticum* with antileishmanial and immunomodulatory activities. Bioorg. Med. Chem. Lett., 15: 4543-4546.

Morton, J.J.P. and M.H. Melone, 1996. Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Intl. Pharmacodyn, 6: 117-119.

Mukerjee, P.K., R. Verpoorte and B. Suresh, 2000. Evalution of *in vivo* wound healing activity of Hyperiicum Patulum (family, hypericaceae) leaf extract on different wound models in rats. J. Ethnopharmacol., 70: 315-321.

Rao, C.V., B. Ravishankar and S. Mehrotra, 2004. Anti-inflammatory and antinociceptive activity of the water decoction *Desmodium gangeticum*. J. Ethnopharmacol., 95: 259-263.

Rao, G.V., H.G. Shivakumar and G. Parathasarathi, 1996. Influence of aqueous extract of centella asiatica (brahmi) on experimental wounds in albino rats. Ind. J. Pharmacol., 28: 249-253.

Singh, N., P.K. Mishra, A. Kapil, K.R. Arya, R. Maurya and A. Dube, 2005. Efficacy of *Desmodium gangeticum* extract and its fractions against experimental visceral leishmaniasis. J. Ethnopharmacol., 98: 83-88.

Taranalli, A.D. and I.J. Juppest, 1996. Study of wound healing activity of seeds of *Trigonella foenum graceum* in rats. Ind. J. Pharm. Sci., 58: 117-119.

The Wealth of India, 1996. A dictionary of Indian Raw Material and Industrial product. New Delhi: Publication and Information Directorate CSIR, 3: 211-213.

Woessner, J.F., 1961. The determination of hydroxyl proline in tissue and protein samples containing small proportion of these amino acids. Arch. Biochem. Biophys., 93: 440-447.

Yeo., J.H., K.W. Lee, H.C. Kim, Y. Lyun and S.Y. Kim, 2000. The effect of pva/chitosan/fibran blended spongy sheets on wound healing in rats. Biol. Pharm. Bull., 23: 1220-1223.