

# Wound healing activity of *Argyrea nervosa* leaves extract

AK Singhal, H Gupta<sup>1</sup>, VS Bhati

MJRP College of Healthcare and Allied Sciences, MJRP University, <sup>1</sup>School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India

## ABSTRACT

**Background:** *Argyrea nervosa* (Convolvulaceae) plant is an example of hallucinogenic plant. The antiseptic, anti-inflammatory, antispasmodic, antibacterial, antiviral, antifungal, anticonvulsant, nootropic, antifertility and aphrodisiac properties have already been reported for this plant. **Aim:** The aim of present work was to evaluate the wound healing property in normal and diabetic animals by oral and topical administration of ethanolic extract of leaves. **Materials and Methods:** Phytochemical investigations showed the presence of various biochemicals (alkaloids, flavonoids, carbohydrates, triterpenoids, proteins, saponins, steroids, tannins). A single injection of alloxan monohydrate (120 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5) was administered to produce diabetes in rats and mice, after overnight fasting. Excision wounds (sized 300 mm<sup>2</sup> and of 2 mm depth) were used for the study of rate of contraction of wound and epithelization. The means of wound area measurement between groups at different time intervals were compared using one-way analysis of variance (ANOVA), followed by Dunnet's test. **Results:** Extracts of *A. nervosa* showed significant wound healing effect in normal (topically treated) and diabetic (both topically and orally treated) rats. In diabetic rats, the topically treated group showed more significant effect than the orally treated groups. **Conclusion:** The present study demonstrates that *A. nervosa* leaves extract applied topically promotes healing of wounds more significantly as compared to oral application, in both normal rats and alloxan induced diabetic rats, where healing is otherwise delayed.

**Key words:** Alloxan, diabetes, wound healing

## INTRODUCTION

*Argyrea nervosa* (family: Convolvulaceae) plant is also known as elephant creeper or woolly morning glory. This plant is found on river banks, edges of lakes and as undergrowth in semi-deciduous forests. Leaves of *A. nervosa* mainly contain  $\beta$ -sitosterol, 1-tricontanol and quercetin. Traditionally, the plant has been used therapeutically for its wide range of clinical effects such as antiviral, antibacterial, anti-fungal and anti-inflammatory properties. It has rejuvenating, age sustainer and spermatogenic activities as well.<sup>[1]</sup> The seeds contain the highest concentration of psychoactive compounds in the entire

plant. In India, usually leaves and root parts of the plant are used as antiseptic and anti-inflammatory drugs.

In Unani system of medicine, its roots are known to possess aphrodisiac and diuretic properties, and have been used to treat gonorrhoea; while its leaves are antiphlogestic, emollient, local stimulant, rubefacient and vesicant. Internally, the extract of *A. nervosa* leaves has been used to cure the boils and swellings. Externally, it is applied on eczema, etches, ringworm infections and skin diseases. Its seeds are used as hypotensive, spasmolytic and tonic.<sup>[2]</sup> 50% ethanolic extract of the seeds in a preliminary biological screening showed antispasmodic activity in the isolated guinea pig ileum<sup>[3]</sup> and antibacterial activity against *Staphylococcus aureus*. The alcoholic extract of the root exhibited statistically significant anti-inflammatory activity against granuloma technique in albino rats.<sup>[4]</sup> The seed-oil showed antifungal activity against *Aspergillus flavus* and *Alternaria solani*.<sup>[5]</sup> A paste of its tubers is applied externally in abscesses of stomach. *A. nervosa* Burm. has been reported for its anticonvulsant activity<sup>[6]</sup> and nootropic activity.<sup>[7]</sup>

Diabetic wound healing is an enigmatic and debilitating complication and poses a serious challenge in the clinical

Address for correspondence: Mr. Hemendra Gupta,  
School of Pharmaceutical Sciences, Jaipur National University,  
Jaipur, Rajasthan, India, E-mail: Hemendra\_gupta@gmail.com

Access this article online	
Quick Response Code:	Website: www.ijabmr.org
	DOI: 10.4103/2229-516X.81978

practice. The exact pathogenesis of the poor wound healing with the diabetic wound is not clearly understood, but evidence from studies involving both human and animal models reveal several abnormalities in the various phases of wound healing process.<sup>[8]</sup> Taking a clue from the antibacterial and anti-inflammatory activity of the plant leaves as reported in the traditional literature, the present study was planned to evaluate the wound healing property of its ethanolic extract, using normal and diabetic rats.

## MATERIALS AND METHODS

### Collection and identification

The plant leaves of *A. nervosa* were collected locally from Kapoor Chand Kulish Smriti Van, Jaipur, Rajasthan, and identified in the Botanical Survey of India/Arid Zone Circle, Jodhpur. The voucher specimen of the same was also deposited at the above-mentioned herbarium (specimen number JNU/JPR/PC/HG-1). Alloxan monohydrate was purchased from Spectrchem Pvt. Ltd. Company, Mumbai, India.

### Selection of animals

Male Wistar rats weighing 150–200 g were used in the study, after obtaining the approval of the Institute's Animal Ethics Committee (approval code no. 005/2009/CPCSEA/JNU). Animals were fed on a standard pellet diet and water ad libitum and maintained at 24–28°C temperature and relative humidity (30% - 70%). Animals marked as fasted were deprived of food for 16 hours, but had free access to water.

### Preparation of the extract

The freshly collected leaves were shade-dried and pulverized using a mechanical grinder. The powdered leaves were macerated with 90% ethanol for 3 days, with occasional shaking. The extract was subjected to preliminary phytochemical tests and percentage yield was calculated in the extract after drying.<sup>[9]</sup>

### Phytochemical screening

Phytochemical screening was carried out to identify the presence of alkaloids, carbohydrate, glycoside, flavonoids, triterpenoids, protein, saponins, steroids, tannins, etc. in the ethanolic extract of *A. nervosa*.<sup>[10]</sup>

### Vehicles

Alloxan was diluted in normal saline and injected intraperitoneally (i.p.). Plant extract and standard drug were suspended in 1% v/v Tween-80 and administered orally (p.o.) to animals with the help of oral feeder. 15% (w/w) extract-ointment was prepared with simple ointment formulation and was applied topically.

### Wound healing activity (normal and diabetic rats)

Rats were made diabetic by a single injection of alloxan

monohydrate (120 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5), after overnight fasting. Blood was drawn from the tail vein 24 hours after the injection and the glucose level was estimated using glucometer (Johnson and Johnson, Mumbai, India). Wounds were made on the rats showing elevated blood glucose level (>250 mg/dl). Blood glucose levels were estimated at the time of the creation of the wounds.

### Excision wound creation

Excision wounds were used for the study of rate of contraction of wound and epithelization. All wounds were of full-thickness type, extending up to the adipose tissue. Animals were anesthetized with slight vapor inhalation of diethyl ether and the back side of each rat was shaved. Excision wounds sized 300 mm<sup>2</sup> and of 2 mm depth were created along the markings using toothed forceps, a surgical blade and pointed scissors. Animals were closely observed for any infection, and those which showed any sign of infection were separated, excluded from the study and replaced. The treatment was done both topically and orally. The extract was applied in a dose of 100 mg/kg/day for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker. The recorded wound areas were measured on a graph paper.<sup>[11-15]</sup>

### Sub-grouping of animals in normal and diabetic rat groups

Group I: Control group, i.e. rats treated neither with extract nor with standard

Group II: Rats treated topically with standard drug ointment, i.e. mupirocin ointment (2% w/w)

Group III: Rats treated topically with extract-ointment (15% w/w) of *A. nervosa* leaves

Group IV: Rats treated orally with ethanolic extract (200 mg/kg, p.o.) of *A. nervosa* leaves

### Statistical analysis

The means of wound area measurement and percentage closure of wounds between groups at different time intervals were compared using one-way analysis of variance (ANOVA), followed by Dunnet's test. Data were analyzed using the Graph Pad Software (5.0 – demo version) and *P* value of <0.05 was considered to be significant.

### Percentage wound closure

Percentage wound closure was calculated by using following formula:

$$\text{Percentage wound closure} = \frac{(\text{Initial area of wound} - n^{\text{th}} \text{ day area of wound})}{(\text{Initial area of wound})} \times 100$$

## RESULTS

The percentage yield of the alcoholic extract of *A. nervosa* leaves was found to be 30% w/w. Qualitative test showed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, proteins, saponins, steroids and tannins. An acute toxicity study on female rats showed no mortality and unusual effects at a dose of 2000 mg/kg, during a time period of 14 days. This helped to predict its non-toxicity and safety.

### Wound healing in normal rats

In normal animals, the animals treated topically with the extract (group III) showed significantly more ( $P < 0.05$ ) wound healing as compared to control animals (group I) on the 8th and 16<sup>th</sup> days. The standard drug treated animals (group II) showed significant ( $P < 0.05$ ) wound healing on the 4th, 8th and 16<sup>th</sup> days, compared to the animals of the control group. Effect of the extract given orally (group IV) was nonsignificant compared to the control animals [Table 1].

The wound closure was 92.96% with the extract ointment (group III) and 94.94% with 2% mupirocin (group II) compared to 80.27% in the control group (group I) on the 16th day of treatment, with a statistically significant difference. Wounds of animals treated orally with the extract did not show any significant closure at all phases [Table 2].

### Wound healing in diabetic rats

In diabetic animals, the extract treated groups III and IV (topically and orally, respectively) showed significant ( $P < 0.01$ ) wound healing as compared to control animals on the 16<sup>th</sup> day, which was comparable to standard mupirocin (group II) treated animals [Table 3].

The animals treated with ethanolic extract topically (group III) showed more wound closure (76.20%) than orally treated (group IV, 74.36%) animals, with a statistically significant difference compared to normal on all days [Table 4].

## DISCUSSION

Wound healing is characterized by three stages, viz., inflammation, proliferation, and remodeling. The proliferative phase typically demonstrates angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. In angiogenesis, new blood vessels grow from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new provisional extracellular matrix by excreting collagen and fibronectin. In epithelialization, epithelial cells crawl across the wound bed to cover it. Fibronectin, the major glycoprotein secreted by

**Table 1: Combined wound area of normal rats (in mm<sup>2</sup>)**

Day	Group I	Group II	Group III	Group IV
0	217.8 ± 18.23	217.1 ± 13.18	211.9 ± 13.25	222 ± 9.946
4	159.1 ± 20.17	81.27 ± 12.04*	111.6 ± 10.76	130.9 ± 11.15
8	73.16 ± 7.58	37.68 ± 7.37*	35.01 ± 5.18*	77.97 ± 11.54
16	42.97 ± 5.24	10.98 ± 2.79*	14.91 ± 4.05*	23.7 ± 4.45

All values are in mean ± SEM \* $P < 0.05$

**Table 2: Percentage wound closure in normal rats**

Day	Group I (%)	Group II (%)	Group III (%)	Group IV (%)
0	-	-	-	-
4	26.95	62.57	47.83	41.01
8	66.40	82.64*	83.48*	64.88
16	80.27	94.94*	92.96*	89.32

\* $P < 0.05$  (compared to normal)

**Table 3: Combined wound area of diabetic rats (in mm<sup>2</sup>)**

Day	Group I	Group II	Group III	Group IV
0	238.5 ± 14.05	238.5 ± 14.05	238.5 ± 14.05	228.4 ± 18.88
4	189.5 ± 26.09	151.8 ± 18.21	142.9 ± 10.04	133.6 ± 10.76
8	119.5 ± 11.3	105.9 ± 12.19	81.27 ± 12.04	79.44 ± 8.17
16	92.1 ± 15.72	46.16 ± 5.98*	56.76 ± 6.795*	61.16 ± 5.98*

All values are in mean ± SEM \* $P < 0.01$

**Table 4: Percentage wound closure in diabetic rats**

Day	Group I (%)	Group II (%)	Group III (%)	Group IV (%)
0	-	-	-	-
4	20.55	36.35*	40.08*	43.98*
8	49.90	55.60*	65.92*	66.69*
16	61.38	80.65*	76.20*	74.36*

\* $P < 0.05$  (compared to normal)

fibroblasts, has important functions of chemo-attraction for macrophages, fibroblasts and endothelial cells, promoting re-epithelialization and acting as a transduction agent in wound contraction. Wound contraction occurs by myofibroblasts, which establish a grip on the wound edges, bringing them in apposition.

The present study concluded that the topical application of ethanol extract of *A. nervosa* leaves plays a major role in wound healing in normal and diabetic animals. It was also found that the ethanolic extract of *A. nervosa* is more effective topically compared to oral preparation. The present study also demonstrates that *A. nervosa* leaf extract applied topically promotes healing of wound in alloxan-induced diabetic rats, where healing is otherwise delayed. These preliminary results can further be used as a basis for a full-fledged study to evaluate the role of extract of *A. nervosa* in diabetic animal models, so as to elucidate its role in the treatment of diabetic foot.

## REFERENCES

1. Gupta DP. The Herbs. 1st ed. Indore: Smt. Prem Lata Gupta Prakashak;

2008. p. 57-8.
2. Dhiman AK. Ayurvedic Drug Plants. Delhi: Daya Publication House; 2006. p. 331-2.
  3. Bhakuni DS, Goel AK, Jain S, Mehrotra BN, Srimal RC. Screening of Indian plants for biological activity. *Indian J Exp Biol* 1990;28:619-37.
  4. Srivastava MC, Kant V, Tewari JP. Antiinflammatory activity of roots of *Argyrea nervosa*. *Mediscope* 1972;15:219-22.
  5. Mishra SH, Chaturvedi SC. Antibacterial and antifungal activity of the oil and unsaponifiable matter of *Argyrea nervosa*. *Indian Drugs Pharm* 1978;13:29-30.
  6. Vyawahare NS, Bodhankar L. Anticonvulsant activity of *Argyrea nervosa* in mice. *Indian J Pharm Sci* 2009;71:131-4.
  7. Joshi H, Kaur N, Chauhan J. Evaluation of Nootropic Effect of *Argyrea nervosa* in Mice. *J Health Sci* 2007;53:382-8.
  8. Nayak S. Influence of Ethanol Extract of *Vinca rosea* on Wound Healing in Diabetic Rats. *J Biol Sci* 2006;6:51-5.
  9. Khandelwal KR. Practical Pharmacognosy. 16<sup>th</sup> ed. Pune: Nirali Prakashan; 2006. p. 86-9.
  10. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy. 26<sup>th</sup> ed. Pune: Nirali Prakashan; 2006. p. 593-7.
  11. Ghosh MN. Fundamentals of Experimental Pharmacology. 3<sup>rd</sup> ed. Kolkata: Hilton and Company; 2005. p. 190-7.
  12. OECD. Test No 420: Acute oral toxicity – Fixed dose procedure OECD Guideline for Testing of Chemicals, Section 4: Health Effects. Available from: [http://www.oecd-ilibrary.org/environment/test-no-420-acute-oral-toxicity-fixed-dose-procedure\\_9789264070943-en;jsessionid=b49ldj89cub1j.delta](http://www.oecd-ilibrary.org/environment/test-no-420-acute-oral-toxicity-fixed-dose-procedure_9789264070943-en;jsessionid=b49ldj89cub1j.delta). [Last cited on 2010 Dec 07].
  13. Vogel HG. Drug Discovery and Evolution Pharmacological Assays. 2nd ed. Springer: Germany; 2002. p. 940-1051.
  14. Manjunatha BK. Evaluation of wound healing potency of *Vernonia arborea*. *Indian J Pharmacol* 2005;37:223-6.
  15. Nayak BS, Anderson M, Pinto Pereira LM. Evaluation of wound-healing potential of *Catharanthus roseus* leaf extract in rats. *Fitoterapia* 2007;78:540-4.

**How to cite this article:** Singhal AK, Gupta H, Bhati VS. Wound healing activity of *Argyrea nervosa* leaves extract. *Int J App Basic Med Res* 2011;1:36-9.

Source of Support: Nil. **Conflict of Interest:** None declared.

### Author Help: Online submission of the manuscripts

Articles can be submitted online from <http://www.journalonweb.com>. For online submission, the articles should be prepared in two files (first page file and article file). Images should be submitted separately.

1) **First Page File:**

Prepare the title page, covering letter, acknowledgement etc. using a word processor program. All information related to your identity should be included here. Use text/rtf/doc/pdf files. Do not zip the files.

2) **Article File:**

The main text of the article, beginning with the Abstract to References (including tables) should be in this file. Do not include any information (such as acknowledgement, your names in page headers etc.) in this file. Use text/rtf/doc/pdf files. Do not zip the files. Limit the file size to 1024 kb. Do not incorporate images in the file. If file size is large, graphs can be submitted separately as images, without their being incorporated in the article file. This will reduce the size of the file.

3) **Images:**

Submit good quality color images. Each image should be less than **4096 kb (4 MB)** in size. The size of the image can be reduced by decreasing the actual height and width of the images (keep up to about 6 inches and up to about 1800 x 1200 pixels). JPEG is the most suitable file format. The image quality should be good enough to judge the scientific value of the image. For the purpose of printing, always retain a good quality, high resolution image. This high resolution image should be sent to the editorial office at the time of sending a revised article.

4) **Legends:**

Legends for the figures/images should be included at the end of the article file.