Original Article

Evaluation of wound healing potential of *Pterocarpus marsupium* heart wood extract in normal and diabetic rats

Abstract

Aim: The aim of the present study is to evaluate and compare the cutaneous wound healing potential of *Pterocarpus marsupium* in normal and diabetic rats and make inference for the cutaneous wound healing potential by possible "mode of action" *P. marsupium* extract. **Materials and Methods:** The effect of heart wood extract of *P. marsupium* on wound healing has been studied in diabetic and normal animals. The effect has also been compared with standard (mupirocin ointment) application. In the absence of specific animal model for cutaneous diabetic wound healing, we have used common model of wound healing (excision wound model) in animals having diabetes (by administration of alloxan monohydrate 120 mg/kg i.p.). **Statistics Analysis:** Data were analyzed by using one-way ANOVA, followed by Tukey's post hoc tests, using the Graph Pad Software (5.0 demo version), and *P* value <0.05 was considered to be significant. **Results and Conclusion :** Rats treated with 200 mg/kg/day of *P. marsupium* heart wood extract had high rate of wound contraction, significantly decreased epithelization period, and significant increase in dry weight, wet weight, and hydroxyproline content of the granulation tissue when compared with the diabetic control and normal control groups. Wound contraction together with increased tensile strength and hydroxyproline content support the use of *P. marsupium* heart wood extract in the management of wound healing in normal and diabetic rats.

Key words:
Hydroxyproline, P. marsupium, wound healing

Introduction

Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities. The collagen content of the skin is decreased as a result of reduced biosynthesis and/or accelerated degradation of newly synthesized collagen. These abnormalities contribute to the impaired wound healing observed in diabetes.[1] Wound healing is a complex process involving a highly regulated series of biological events. These include a set of co-ordinated interactions between cells in the dermis and the epidermis, and important relationships have been found to exist between fibroblasts, keratinocytes, and resident dermal cells.[2,3] The heart wood extract of plant *Pterocarpous marsupium* has been established in diabetic management. There is no significant work done in management of diabetic wound complication of the plant *P. marsupium***.** Hence, this study is undertaken to evaluate scientifically.

Plant *P. marsupium was* selected for the study due to its easy availability and wide geographical distribution of the plant globally in almost every climatic condition. *P. marsupium* is been used for treatment of diabetes traditionally. This study is proposed to evaluate and compare the cutaneous wound healing potential of *P. marsupium* in normal and diabetic rats and make inference for the cutaneous wound healing potential by possible "Mode of action" *P. marsupium* extract.

Materials and Methods

Plant materials

The plant materials (heart wood) of *P. marsupium*

Access this article online	
Website: http://www.cysonline.org	Quick Response Code
DOI: 10.4103/2229-5186.94313	

Anil Kumar Singhal, Manish Gupta[1], Sheeja Edwin[1], Rupesh Soni[1]

Venus Medicine Research Center, Venus Remedies Ltd. Baddi,
[1]Department of Pharmacology, B.R. Nahata College of Pharmacy, Mandsaur, Madhya Pradesh, India

Address for correspondence:
Mr. Manish Gupta
Department of Pharmacology, B.R. Nahata College of Pharmacy, Mandsaur, Madhya Pradesh, India.
E-mail: manish_pharma06@yahoo.co.in

were collected from market in Mandsaur (MP). The authentification was done by Dr. Gyanendra Tiwari (Asst. Prof./Scientist, Dept. of Medicinal Plant Science, K. N. K. College of Horticulture, Mandsaur). A voucher specimen no. BRNCP/P/004/2006 was submitted in the herbarium of the Department of Pharmacognosy, BRNCP.

Chemicals

Alloxan, glucose, gluco strips, mupirocin ointment (2% w/w), diethyl ether, ethanol, sterilized cotton, hydroxyl proline, and ehlrich reagent were used.

Methods

Extraction

The heart wood crude herb of *P. marsupium* was coarsely powdered. Soxhlet extraction method was followed for the extraction. A hydroalcholic solvent was used (i.e., 50% ethanol and 50% water) for extraction. Oven-dried coarsely powdered heart wood of *P. marsupium* material was weighed accurately and placed in Soxhlet extraction chamber which was suspended above the flask containing solvent and below a condenser. The flask was heated and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded at certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the hydroalcholic extract was removed and solvent was evaporated by using rotary evaporator. The weight of the residual extract was measured and percent yield was calculated. The % yield was calculated for each extract after drying.[4]

Experimental procedures

Procurement and selection of animals

Wistar rats weighing between 100 and 150 g of either sex were obtained from BRNCP Mandsaur animal house. The animals were stabilized for 1 week; they were maintained in standard condition at room temp, 60 ± 5% relative humidity, and 12-h light–dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output.

Selection of doses

For the assessment of cutaneous wound healing activity, dose level was chosen in such a way that dose was approximately one-tenth of the maximum dose during acute toxicity studies (200 mg/kg).

Induction of diabetes

Rats were made diabetic by a single injection of alloxan monohydrate (120 mg/kg, i.p.) prepared in normal saline after overnight fasting. Blood was drawn from the tail vein after 72 hr the injection, and the glucose levels were estimated using Glucometer (Accu-Chek). Wounds were made on the rats showing elevated blood glucose (>250 mg/dl). Blood glucose levels were estimated at the time of creation of excision wounds.

Preparation of stearic acid ointment

20% (w/w) extract ointment was prepared with stearic acid (base ointment) formulation.

Steps followed for 100 g ointment preparation

0.7 g KOH was dissolved in 60 ml water, and then 20 g glycerine and 10 g of *P. marsupium* extract were added. In other beaker, 24 g stearic acid was melted and mixed in a second beaker with constant stirring.

Excision wound model

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 di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300 mm^2 and 2 mm depth were made by cutting out piece of skin from the shaven area. The entire wound was left open. Animals were closely observed for any infection and those which showed any sign of infection were separated, excluded from study, and replaced. The treatment was done topically in all the cases. The extract was applied 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. Recording of wound areas was measured on graph paper. The day of scar falling, after wounding without any residual raw wound, was considered as the day of epithelization.[5,6]

Grouping of animal

Group I : Diabetic wound controls were applied topically with base ointment

Group II : Diabetic wounds were applied topically with standard drug ointment, i.e., mupirocin ointment (2% w/w)

Group III : Diabetic wounds were applied topically with extract ointment (20% w/w) of *P. marsupium*

Group IV : Normal wound controls were applied with base ointment

Group V : Normal wounds were applied topically with standard drug ointment, i.e., mupirocin ointment (2%w/w)

Group VI : Normal wounds were applied topically with extract ointment (20% w/w) of *P. marsupium.*

Incision wound healing activity in diabetic rats

A longitudinal paravertebral incision of 6 cm in length was made through the skin and cutaneous muscle on the back in anesthetized rats. After the incision, surgical sutures were applied at intervals of 1 cm. The wounds were left undressed

(day 0). The sutures were removed on the 8th postwound day and the application of extract was continued. The skin-breaking strength was measured on the 11th day by tensiometer.[5,6]

Tensiometer structure

A tensiometer was designed consisting of a 6–12 inch wooden board with a 4-inch long arm, fixed on each side of longest possible distance of board and a platform in middle of board. A pulley with a bearing was mounted on the top of one arm. A coassembly consisting a reservoir with water and a plastic bottle was also designed.

Method applied for measuring tensile strength

To measure the tensile strength, the board was placed at the edge of table, the rats were again anesthetized, and each rat was placed on wooden platform situated on the middle of the board. The thickness of platform was adjusted in a manner such that the wound was on the same level as the tips of the arms. The clamps were then carefully clamped on the skin of the opposite edges of the wound.

The position of the board was adjusted so that the bottle received a rapid and continuous flow of water from a large reservoir, until the wound began to open. The weight of water required to open the wound was measured in grams and considered as tensile strength.

Grouping of animals

Group I: Diabetic wound controls treated orally with normal saline

Group II: Diabetic wounds treated orally with 200 mg/kg extract

Group III: Normal wound controls treated orally with normal saline

Group IV: Normal wounds treated orally with 200 mg/kg extract.

Dead space wound healing activity in diabetic rats

Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on left side in the groin and axilla on the ventral surface of each rat. On the 10th postwounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the weight of the granulation tissue, the tissue was dried at 60°C for 12 hr, and the dry granulation tissue weight was recorded. To the dried tissue 5 ml 6N HCL was added and kept at 110°C for 24 hr. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline.[7]

Grouping of animals

Group I: Diabetic wound controls treated orally with normal saline

Group II: Diabetic wounds treated orally with 200 mg/kg extract

Group III: Normal wound controls treated orally with normal saline

Group IV: Normal wounds treated orally with 200 mg/kg extract

Statistical analysis

The means of wound area measurements, epithelization period, tensile strength, wet and dry weight, and hydroxyproline content of the granulation tissue between groups were compared using a one-way ANOVA, followed by Tukey's *post hoc* tests. Data were analyzed using the Graph Pad Software (5.0 demo version), and P value <0.05 was considered to be significant.

Results

Percentage yield

The percentage yield obtained for hydroalcoholic extract of *P. marsupium* heart wood was 30% w/w.

Wound healing studies

During study of cutaneous wound healing in normal and diabetic rats, following results were obtained: [Table 1, Figure 1].

In diabetic animals, the extract-treated group III showed significantly greater wound healing as comparative to diabetic control animals group I; in normal animals, the extract-treated group VI showed significantly greater wound healing when compared with normal control animals group IV. The

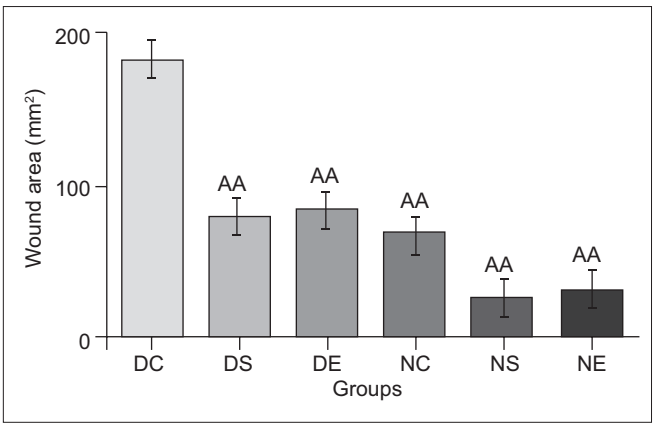

Figure 1: 16th day wound area

Table 1: Excision wound model (wound area, mm²)

Day	Group I DC	Group II DS	Group III DE	Group IV NC	Group V NS	Group VI NE
16	183.33±5.110	79.167±4.362**	84.167±3.745**	68.33±2.472**	27.50±2.141**	30.83±2.386**

The values are in mean±SEM. **Very significant $P<0.001$; *Significant $P<0.05$

standard drug-treated animals in both normal and diabetic animals were showed significantly greater wound closure when compared with control and extract-treated animals.

Percentage wound closure

Percentage wound closure =

$$\frac{\text{Initial area of wound} - \text{N}^{\text{th}}\text{day area of wound}}{\text{Initial area of wound}} \times 100$$

In diabetic animals, the extract-treated group III (61.0%) showed greater percentage wound closure as comparative to diabetic control group I (25.17%). In normal animals, the extract-treated group VI (83.48%) showed greater percentage wound closure when compared with normal control animals group IV (76.22%). The standard drug-treated animals in both normal and diabetic animals showed greater percentage wound closure diabetic standard group II (54.76%) and normal standard group V (87.86%) when compared with control and extract-treated animals [Table 2].

Incision wound model

Tensile strength (gm)

In diabetic animals, the extract-treated group II showed significantly greater tensile strength when compared with diabetic control animals group I. In normal animals, the extract-treated group IV showed significantly greater wound healing when compared with normal control animals group III [Table 3, Figure 2].

Dead space wound model

Wound parameters

In diabetic animals and normal animals, the extract-treated groups II and IV showed significantly higher levels of hydroxyproline when compared with control animals group I and III. A significant increase was also observed in the dry and wet weight of the granulation tissue in the extract-treated groups II and IV when compares with control animals groups I and III [Table 4, Figures 3-6].

Discussion

Granulation collagen maturation and scar formation are some of phases of wound healing which run concurrently but independent of each other. Wound contraction, epithelization, and fibrosis are the biological response regulated by the body on cellular defence mechanism.

The faster wound contraction rate may be due to stimulation of interleukin (an inflammatory α-chemokinin). It may increase gap junctional intracellular communication in

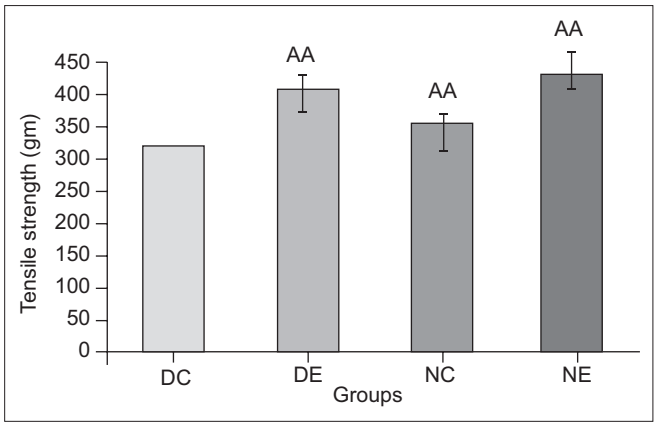

Figure 2: Tensile strength of histogram

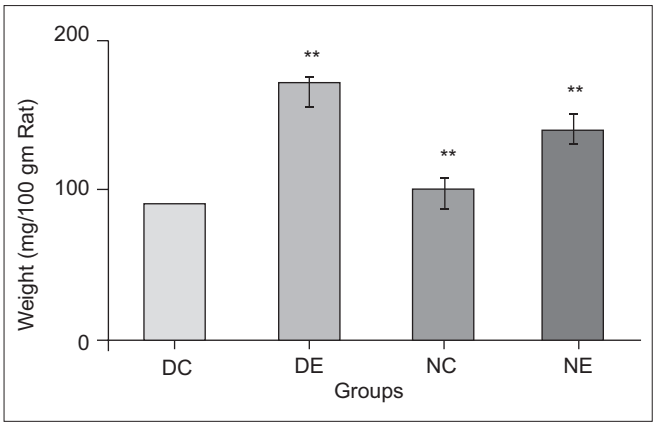

Figure 3: Wet granulation histogram

Table 2: Percentage wound closure

Groups day	Group I DC	Group II DS	Group III DE	Group IV NC	Group V NS	Group VI NE
0	0	0	0	0	0	0
4	16.0	9.52	12.35	10.78	22.42	17.86
8	20.74	19.04	19.69	25.36	35.68	35.27
6	25.17	54.76	61.0	76.22	87.86	83.48

Table 3: Tensile strength (gm)

Group I	Group II	Group III	Group IV
DC	DE	NC	NE

N = 6; ***P* < 0.001; data expressed in mean ± SEM

Table 4: Wound parameters

Group	Group I	Group II	Group III	Group IV
Parameter	DC	DE	NC	NE

N = 6; ***P* < 0.001; data expressed in mean ± SEM

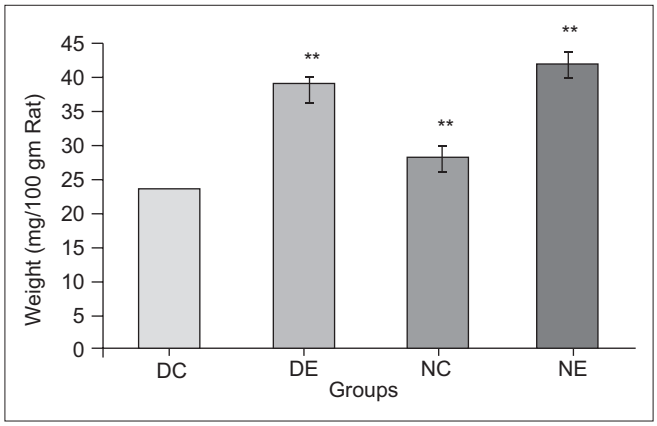

Figure 4: Dry granulation histogram

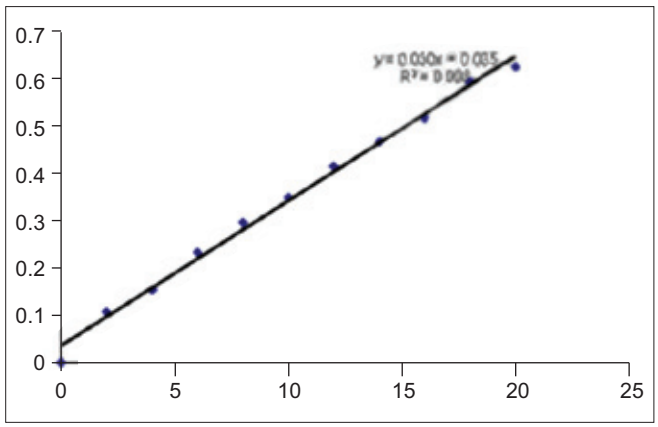

Figure 5: Standard curve of hydroxylproline

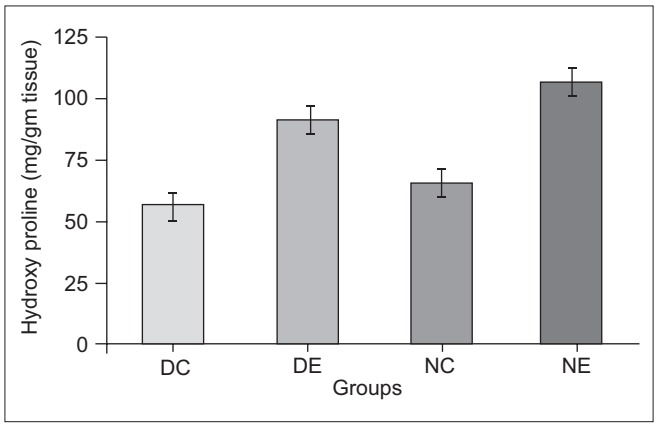

Figure 6: Hydroxyproline histogram

cultured fibroblasts and induce a more rapid maturation of granulation tissue.[8]

Molecular oxygen plays an important role in pathogenesis and therapy of chronic wounds. Overproduction of reactive oxygen species results in oxidative stress, thereby causing cytotoxicity and delayed wound healing.[9]

The heart wood extract of *P. marsupium* has been shown to possess steroids, flavanoids, and phenolic compound on preliminary screening. The wound closure is mainly dependent on the formation of collagen and maturation of collagen. This may be because of flavanoids and steroids which are responsible for free radical scavenging activity and help to promote most important phase of wound healing. The increase in wet and dry granulation tissue weight suggests higher cellular migration and deposition of collagen, proteins, and other cells at the site of wound and provide strength to wound. The high hydroxyproline content suggests greater deposition of collagen because hydroxyproline is the constitute of collagen. In diabetic patient, delay in wound healing may be due to high blood glucose level, denaturation of proteins and cellular components by high blood glucose, and pressure of free radicals, especially oxidative free radicals. The heart wood extract of *P. marsupium* showed anti-hyperglycemic,[10] hypoglycemic,[11] and antifungal activity[12] which are due to various active phytochemicals present in the plant extract, i.e., steroids, phenolic, and flavanoids. Hence, on the basis of results obtained and above facts, we can say that the faster wound healing activity of heart wood extract of *P. marsupium* in diabetic and normal animals may be due to the presence of phytochemicals and their effect on components of wound healing.

Conclusion

The effect of heart wood extract of *P. marsupium* on wound healing has been studied in diabetic and normal animals. The effect has also been compared with standard (mupirocin ointment) application. In the absence of specific animal model for cutaneous diabetic wound healing, we have used common model of wound healing (excision wound model) in animals having diabetes (by administration of alloxan monohydrate 120 mg/kg i.p.).

In the excision wound model, the drug extract was made into an ointment in stearic acid base and applied topically after creation of wound in diabetic and normal animals. Measurement of wound contraction was done 4th, 8th, and 16th day of wound creation. The results show that application of heart wood extract of *P. marsupium* significantly increased wound healing in both normal and diabetic animals. In the incision wound model, the tensile strength was measured on day 11 with the help of tensiometer. The results show that application of heart wood extract of *P. marsupium* significantly increased tensile strength in both normal and diabetic animals. In the dead space wound model, the dry and wet weight of granulation tissue and hydroxyproline content were measured on 10th postwounding day. The results show that application of heart wood extract of *P. marsupium* significantly increased dry and wet weight and hydroxyproline content in both normal and diabetic animals.

On the basis of above, it may be conclude that plant extract promotes wound healing in both diabetic and normal

animals by topical application of extracts. This supports its prevalent use in treatment of diabetes and infection; however, furthermore specific and controlled biophysical and biochemical studies are required to draw a definite conclusion, and this was not possible in this study due to limited availability of resources.

References

1. Brem H, Canic MT. Cellular and molecular basis of wound healing in diabetes. J Clin Invest 2007;117:1219-22.
2. Available from: http://www.revolutionhealth.com/conditions/[Last assesed on 2008 Sep 15].
3. Available from: http://www.idexx.com/ [Last Assessed on 2008 Sep 15].
4. Kokate CK, Purohit AP, Gokhale SB. Text book of Pharmacognosy, 26th ed. Pune: Nirali Prakashan; 2006. p. 593-7.
5. Nayak BS, Anderson M, Periara LM. Evaluation of wound healing potency of catharanthus roseus leaf extract in rats. Fitoterapia 2007;78:540-4.
6. Nayak BS, Pinto Pereira L, Muharaj D. Wound healing activity of Carica papaya L. in experimentally induced diabetic rats. indian J Exp Biol 2007;45:739-43.
7. Reddy GK, Enwemeka CS. A simplified method for the analysis of hydroxyproline in biological response. Clin Biochem 1996;29:225-9.
8. Mayer KE, Saggers GC, Ehrlich HP. Effect of IL-8 on granulation tissue maturation. J Cell Phisiol 2002;193:173-9.
9. Dissemond J, Goos M, Wagner SN. The role of oxidative stress in the pathogenesis and therapy of chronic wounds. Hautarzt 2002;53:719-23.
10. Vats V, Grover JK, Rathi SS. Evaluation of antihyper glycemic and hypoglycemic effect of Trigonella foenum graceum Linn. And *Pterocarpous marsupium* Linn. in normal and alloxanized diabetic rats. J Ethnopharmacol 2002;79: 95-100.
11. Ahmed F, Khalid P, Chaubey M, Restogi AK, Kidwai JR. Hypoglycemic activity of *Pterocarpous marsupium* wood. J Ethnopharmacol 1991;35:71-5.
12. Dhir GG, Mohan G, Verma BR, Mishra SS. Studied the anti fungal activity of *Pterocarpous marsupium*: A clinically evaluation. Indian J Dermatol Venreol Leprol 1982;48:154-6.

How to cite this article: Singhal AK, Gupta M, Edwin S, Soni R. Evaluation of wound healing potential of *Pterocarpus marsupium* heart wood extract in normal and diabetic rats. Chron Young Sci 2012;3:42-7.

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