

Wound healing activities of Bark Extract of *Jatropha curcas* Linn in albino rats

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ABSTRACT

Objective: To investigate the wound-healing properties of crude bark extract of *Jatropha curcas* Linn in Wistar albino rats.

Methods: This work was carried out in the Department of Biochemistry, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India, in the year 2005. We divided the animals into 3 groups of 12 each. Group I was saline control without wound, group II was animals treated with JC extract in the dose of 2 ml/kg body weight with wound and group III was animals treated with 4 ml/kg body weight with wound. The wound healing parameters like wound breaking strength, epithelization period, percent wound contraction, granulation tissue breaking strength, granulation tissue dry weight, hydroxyproline level and histological features

were assessed by using incision, excision and dead space wound models.

Results: The results obtained indicated that *Jatropha curcas* accelerates the healing process by increasing the skin breaking strength, granulation tissue breaking strength, wound contraction, dry granulation tissue weight and hydroxyproline levels. A significant decrease in epithelization period was also observed. The histopathological examination of granulation tissue showed much advanced phase of healing, with more collagen, which has organized to form bundles.

Conclusion: The results of the study suggest that the crude bark extract of *Jatropha curcas* was very effective in accelerating wound healing process.

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Jatropha curcas Linn (Euphorbiaceae) is commonly known as physic nut, is a big shrub around 6 m tall with spreading branches and stubby twigs. The plant is native of tropical America and also found in Jamaica, India and Brazil. The plant has been used as folk medicine in different countries to treat different ailments in humans. Cameroon natives apply the leaf decoction against arthritis.¹ Colombians drink the leaf decoction against venereal diseases and apply the latex to burns, hemorrhoids, and ulcers. Bahamans drink the decoction against heartburn. Cubans apply the latex for toothache. Leaves are

regarded as antiparasitic, and are also applied against scabies and hard tumors. Some of the ethnomedical uses of the extracts of *Jatropha curcas* leaves and roots include use as a remedy against cancer, as an abortifacient, antiseptic, narcotic, purgative, diuretic and hemostatic. When the crushed leaf of the plant is applied directly to cuts and bleeding wounds, bleeding soon stops.² This plant is a folk remedy for alopecia, burns, syphilis, dermatitis, inflammation, rash, rheumatism, ulcers, scabies and sores.³ The latex is applied topically to bee and wasp stings and also used to dress sores and ulcers.¹ Furthermore,

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Villegas *et al*⁴ have demonstrated a significant wound healing activity of *Jatropha curcas* extracts. A survey of literature revealed that no systematic approach has been made to study the wound healing activity of this plant. The present study was undertaken to assess the effect of this indigenous plant on different parameters related to wound healing in rats.

Methods. The bark of *Jatropha curcas* Linn, is collected from the local areas of Udupi district, and authenticated by Professor Anni Shirwaikar, Manipal College of Pharmaceutical Sciences, Manipal. A voucher specimen (No. PP524) has been deposited at the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences Manipal. For the experiments, the plant bark was cut into small pieces and homogenized using a blender mixer. The juice was squeezed, collected and used on the same day. The yield from 100 g of bark was 16 ml. Three deoxypreussomerins namely, palmarumycins CP1, JC1 and JC2 have been isolated from stems of *Jatropha curcas*. The second and third compounds are antibacterial constituents, which were characterized from spectral evidence. The x-ray crystallographic structure of palmarumycin JC1 was also studied.⁵ Healthy Wistar albino rats of either gender, approximately of the same age and weighing about 150-200 g were used for the study. They were fed with standard chow diet (Pranav agro industries Ltd, Sangli, Maharashtra) and water *ad libitum*. They were housed in polypropylene cages, maintained under standard conditions. The experimental protocol was subjected to scrutiny of institutional animal ethical committee for experimental clearance IAEC/KMC/UA/2000. Acute toxicity studies were carried out with healthy albino rats of either gender were divided into 6 groups (n=12) and were fed orally with increasing doses 1, 2, 3, 4, 8, 16 and 32 ml/kg body weight of crude extract. Total crude extract administered orally in doses up to 6 ml/kg body weight did not produce any sign of toxicity or mortality in rats when observed for 14 days after administration. Experimental animals were divided into following 3 groups, each group consisting of 12 albino rats. Group A - untreated (control) rats with wound; Group B - extract treated rats (2 ml/kg body weight orally) with wound; Group C - extract treated rats (4 ml/kg body weight orally) with wound. The studies were carried using ketamine (1 ml/kg body weight), (Made in India by Neon Laboratories Ltd) anesthetized rats in 3 different wound models at 2 different dose levels (2 ml and 4 ml/kg body weight). For the incision wound model (**Figure 1**) 2 paravertebral incisions (6 cm long) were made through the full thickness of the skin on



Figure 1 - Wound models.

either side of the vertebral column of the rat.⁶ Wounds were closed with interrupted sutures, 1 cm apart. The animals were treated daily with extract from zero day to 9th post-wounding day. The sutures were removed on the 7th day. Wound breaking strength was measured on the 10th post-wounding day. The breaking strength was measured by continuous constant water flow technique.⁷ Each anesthetized animal was secured to the operation table and a line was drawn on either side of the wound 3 mm away from the line. Two allice forceps were firmly applied on to the line facing each other. One of the forceps was fixed while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the water flow was arrested and the volume of water collected in the container (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the wound on the contralateral side. The average reading of the group was taken as an individual value of breaking strength. Mean value gives the breaking strength for a given group. Excision wound (**Figure 1**) was created by a circular piece of full thickness (approximately 500 mm²) was cut off from a predetermined area on the back of rat.⁸⁻¹⁰ The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. Each rat was placed in a separate cage. Animals were treated with extract daily from zero day. Wounds were traced on the 1 mm² graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Wound contraction was expressed as percentage of wound area that had healed. Number

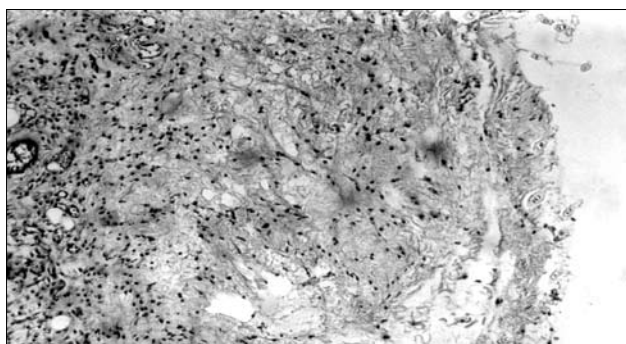


Figure 2 - Collagen deposition in the extract treated group (hematoxylin and eosin stains, pictures taken with Olympus PM 20 photomicroscope 20 X magnification).



Figure 3 - Collagen deposition in the control group (hematoxylin and eosin stains, pictures taken with Olympus PM 20 photomicroscope 20 X magnification).

Table 1 - Effects of bark extract of *Jatropha curcas* (*J.curcas*) on wound healing in incision and dead space wound models.

Wound model/parameters studied	Incision		Dead space	
	Breaking strength (g)	Granulation tissue weight (g/100g) rat	Breaking strength (g)	Hydroxyproline mg/g tissue
Wounded Control	276.16 ± 22.89	30 ± 1.35	285 ± 15.57	14.72 ± 4.02
Crude extract of <i>J.curcas</i> treated 2ml/kg body weight	346.66 ± 58.62*	37.25 ± 2.81*	351 ± 13.70*	20.96 ± 3.96*
Crude extract of <i>J.curcas</i> treated 4ml/kg body weight	387.75 ± 42.39*	42.51 ± 3.28*	396 ± 18.58*	23.75 ± 2.82*
Values are mean ± SD, n=12 rats in each group. *Significant difference from control ($p < 0.05$).				

Table 2 - Effects of bark extract of *Jatropha curcas* on the excision wound model.

Treatment	Epithelisation period (days)	Excision wound model (percentage of wound contraction by days)										
		2	4	6	8	10	12	14	16	18	20	22
Wounded control	21.48 ± 0.42	8.76± 0.58	22.36±1.32	31.75±2.36	46.6±2.37	70.1±3.81	80.65±1.84	86.18±3.31	90.14±3.62	95.39±1.67	97.2±1.52	99.9±0.01
Crude extract of <i>Jatropha curcas</i> treated with 2ml/ kg	18±0.51*	9.17±1.61	23.16±1.41	33.18±1.68	48.7±1.92	71.0±2.85	84.67±2.35	87.1±2.21	94.5±2.71	99.5±0.05		
Crude extract of <i>Jatropha curcas</i> treated with 4ml/kg	16±1.16*	9.91±1.78	24.0±1.54	37.18±2.35	49.6±3.50	72.1±2.36	87.41±3.14	94.3±3.51	99.0±0.08			
Values are mean ± SD (standard deviation), n=12 rats in each group, *Significant difference from control (p<0.05).												

of days required for falling of the eschar without any residual raw wound gave the period of epithelization. Dead space wound models (**Figure 1**) were created by making a small transverse incision in the lumbar region, on either side of the vertebral column, in each animal. Two polypropylene tubes (2.5 x 0.5 cm²) were inserted subcutaneously one on either side of the vertebral column and pushed cephalhead for 3-4 cm for the final implantation to harvest the granulation tissue. The animals were treated with the extract from 0-9th post-wounding day considering wounding day as zero. Granulation tissue formed on the implanted tubes was carefully dissected out on

the 10th post-wounding day and the tensile strength was measured by continuous constant water flow technique.⁷ Mean value gives the breaking strength for a given group. The tissue was dried in an oven at 60°C for 24 hours and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the estimation of hydroxyproline content in the tissue.¹¹ Histopathology were carried out by a small section of the wet tissue was kept in 10% formalin solution for histopathological examinations to determine the pattern of lay-down for collagen using hematoxylin and eosin stains.

Statistical analysis was carried out by comparing all treated groups with the control group. The results were analyzed statistically using one-way analysis of variance method to identify the differences between treated groups and control. The data were considered significant at $p < 0.05$.

Results. In incision wound model, significant increase ($p < 0.05$) was observed in the skin breaking strength on 10th post-wounding day in the animal treated with the both doses of extract (**Table 1**). The extract treated animals of dead space wound model showed significant increase in dry granulation tissue weight, granulation tissue breaking strength and hydroxyproline levels (**Table 1**) at both the dose levels. Histopathological studies revealed increase in collagen deposition in the extract treated group (**Figure 2**) as compared to control (**Figure 3**). The extract treated group showed more advanced phase of healing and better organized bundles of collagen. In studies using excision wound model, animals treated with the bark extract of *Jatropha curcas* showed a significant decrease in epithelization period as evidenced by shorter period for fall of eschar as compared to control. The plant extract also facilitated the rate of wound contraction significantly at both the dose levels (**Table 2**).

Discussion. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is the product of the integrated response of several cell types of injury. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue. Wound healing, complex sequences of events involve 4 phases. (i) Coagulation which prevents blood loss. (ii) Inflammation and debridement of wound. (iii) Epithelial repair, including proliferation, mobilization, migration and differentiation. (iv) Tissue remodeling and collagen deposition. Any agent which accelerates the above process(es) can be termed as a promoter of wound healing. In spite of tremendous advances in the chemical industry, the availability of substances capable of stimulating the process of wound repair is still limited.¹² Plants with wound healing activity have been reported and experimentally studied on various wound models to reveal the most active promising compounds.¹⁰ Results obtained in the present study suggest that treatment of albino rats with the fresh homogenized

crude extract of *Jatropha curcas* has accelerated the wound healing process. Increase in tensile strength may be due to increase in collagen concentration and stabilization of the fibers.¹³ The results suggest that treatment with fresh homogenized crude extract of *Jatropha curcas* may have a beneficial influence on the various phases of wound healing such as fibroplasia, collagen synthesis and wound contraction, resulting in faster healing. These findings partially justify the inclusion of this plant in the management of wound healing in folk medicine. Further experiments are needed to test the effect of this plant in the treatment of chronic wounds.

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