

Original Research Article

Evaluation of wound healing potential of *Jatropha gossypifolia* Linn. root extracts in normal and diabetic rats

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Abstract

Jatropha gossypifolia Linn. is used in Indian folk medicine for treatment of cuts and wounds. Root extract is used to promote wound and fracture healing in animals. Present study was conducted to assess wound healing potential of ointments prepared using successive extracts of roots of the plant. Incision and excision wounds were inflicted upon normal and nicotinamide streptozotocin-induced diabetic rats, respectively. Animals were treated topically with ointments containing 5% and 10% w/w petroleum ether (60-80 C), chloroform, methanol and aqueous extracts. Percentage of wound contraction, epithelization time, tensile strength, hydroxyproline content and histopathology of regenerated skin were evaluated in comparison with reference nitrofurazone (0.2% w/w) ointment. Breaking strength of incision wounds in 5% and 10% w/w methanolic extract ointment treated groups significantly ($P < 0.01$) increased to 695.75 and 759.50 g, respectively, as compared to standard (653.92g). In diabetic rats, apart from methanol extract, aqueous extract also exhibited extraordinary wound healing capacity and elevated hydroxyproline content. Measurement of hydroxyproline, the product of collagen breakdown, is used as an index of collagen turnover. Elevated hydroxyproline content in diabetic animals signify the increased collagen deposition in wounded tissues as evident from histopathology. There is also an increase in the tensile strength of incision wounds in rats. The experimental study confirms the folk medicinal use of the plant.

Keywords: Excision, incision, diabetic wound, hydroxyproline.

Introduction

In India, medicines based on herbal origin have been the basis of treatment for various diseases[1]. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, skin infections, inflammation, leprosy, diarrhoea, scabies, venereal disease, ulcers, snake bite, etc[2]. Traditional Indian system of medicine comprises of many plants with diverse medicinal properties, which require detailed investigation for effective drug development. Plant products are potential agents for wound healing and largely preferred because of their widespread availability, non-toxicity, absence of undesired side effects and their effectiveness as crude preparations[3].

A wound is a type of an injury in which the skin is torn, cut or punctured (an open wound), or where a blunt force trauma causes a contusion (a closed wound). The process of wound healing is related to tissue reconstitution which is the course of action by which the body replenishes the cells that are being lost by normal physiologic events. Healing of an acute wound follows a conventional chain of events. This chain of events occurs in a regulated fashion that is reproducible from wound to wound. The five overlapping phases that characterize wound healing include (a) hemostasis, (b) inflammation, (c) cellular migration and proliferation, (d) protein synthesis and wound contraction, and (e)

remodeling. Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process [4].

Jatropha gossypifolia L. is a perennial ornamental shrub up to 3 m tall, native to Brazil which grows wild in different parts of India[5]. Its various parts are traditionally used in malaria, toothache, anaemia, vertigo, leprosy, diarrhea, dysentery, as antibiotic, emmenagogue, purgative, stomachic, febrifuge, insecticidal, blood purifier, antidote for snake bite, to treat wound, sores and reduce pain[6]. Whole plant and latex of *Jatropha gossypifolia* L. have been ethnobotanically reported for treatment of cuts and wounds [7] and roots have been employed in the treatment of wounds and fractures in animals[8]. The leaves of the plant have been proved to possess anti-hypertensive, antimalarial, antimicrobial and molluscicidal activity. Roots of the plant have exhibited anti-cancer property and seeds have shown molluscicidal. Stem has anti-hypertensive activity; stem bark has anti-inflammatory, anti-viral and molluscicidal activity while stem latex exhibits haemostatic and molluscicidal activity[9]. The present study was carried out to investigate the wound healing potential of *Jatropha gossypifolia* roots using *in vivo* experimental models i.e. linear incision and streptozotocin-induced diabetic circular excision wound.



Material and methods

Plant material and extraction

The roots of *Jatropha gossypifolia* (Euphorbiaceae) were collected from the campus of Guru Jambheshwar University of Science & Technology, Hisar, Haryana, India in October, 2011 from healthy plants and the herbarium so prepared was authenticated by Dr. H. B. Singh (Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India) under voucher specimen no. NISCAIR/RHMD/Consult/-2011-12/1887/187 dated 16-11-2011.

Plant material was dried under shade and coarsely powdered. Dried powder (1 kg) was then successively extracted with petroleum ether (60-80 C), chloroform and methanol using soxhlet apparatus. The material was finally extracted with boiling water. The extracts were concentrated with a rotary evaporator, (40 C), freeze dried and stored in refrigerator until used. Preliminary phytochemical screening was performed as per customary procedure.

Chemicals

Streptozotocin (STZ) was purchased from Sigma chemical company. All other chemicals used in the experiments were purchased locally (Merck or SD fine Chemicals) and were of analytical grade.

Formulation of ointments

Ointment base was prepared using equal parts of poly ethylene glycol 4000 and poly ethylene glycol 400. This served as control and was used to prepare the ointments by fusion method. Dried powdered extracts were added to the ointment base to obtain 5% and 10% (w/w) concentration. Nitrofurazone (0.2% w/w) ointment was used as standard for positive control.

Animals

Wistar rats (150–200 g) of either sex were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India. The animals were housed in polypropylene cages under standard conditions (25±2 C, 12 h light and dark cycle) with free access to standard pellet feed and water *ad libitum*. All the experimental procedures and protocols were approved by the Institutional Animals Ethics Committee (Endst. no. IAEC/89-105, dated 03-12-11) as per CPCSEA guidelines. The animals were used for experimentation after one week of acclimatization period.

Diabetes induction

Diabetes was induced in overnight fasted rats by intraperitoneal injection of freshly prepared solution of streptozotocin (55 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) 15 minutes after intraperitoneal administration of nicotinamide (110 mg/kg body weight). The animals were allowed to drink 20% glucose solution to overcome drug induced hypoglycemia. After 3 days, blood samples were collected retro-orbitally in fluoride tubes from overnight fasted animals and then centrifuged at 6000 rpm for 15 minutes to obtain

plasma for glucose estimation. Glucose levels were estimated using glucose oxidase method and body weight was checked regularly up to 1 week of streptozotocin injection. The animals having marked hyperglycemia (fasting plasma glucose > 200 mg/dl) were selected for the study[10].

Wound healing study

Incision wound model

Animals were anesthetized with intraperitoneal injection of ketamine (50 mg/kg body weight) and their dorsal surface was shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. One full thickness incision of 5 cm length was made through the skin. The parted skin was stitched using 3 interrupted sutures 1 cm apart using black silk thread and curved surgical needle[11]. The animals were divided into ten groups of six each as follows.

- Group 1: Control (treated with ointment base)
- Group 2: Standard (treated with 0.2% nitrofurazone ointment)
- Group 3: Treated with 5% w/w petroleum ether extract ointment
- Group 4: Treated with 10% w/w petroleum ether extract ointment
- Group 5: Treated with 5% w/w chloroform extract ointment
- Group 6: Treated with 10% w/w chloroform extract ointment
- Group 7: Treated with 5% w/w methanol extract ointment
- Group 8: Treated with 10% w/w methanol extract ointment
- Group 9: Treated with 5% w/w aqueous extract ointment
- Group 10: Treated with 10% w/w aqueous extract ointment

Each animal was kept in a separate cage and treated as per grouping for 9 days. The stitches were removed on 9th post wounding day and tensile strength was measured on 10th day by continuous, constant water flow technique [12,13,14].

Diabetic excision wound model

Streptozotocin induced diabetic Wistar rats of either sex were anesthetized with intraperitoneal injection of ketamine (50 mg/kg body weight) and their dorsal surface was shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. One full thickness circular excision wound (14 mm diameter) was created on the dorsal middle line. The animals were grouped as mentioned in incision model and treated topically once a day, starting from wound induction until complete healing. Percentage wound contraction, epithelization time, hydroxyproline content and histopathology of regenerated skin were evaluated.

The progressive decrease in wound area was monitored periodically at every third day by taking photographs and tracing the wound boundaries on a transparent paper to measure the wound areas in all the groups[15]. After complete epithelization, all the animals were euthanized using diethyl ether and used to determine hydroxyproline content. The wound tissues were excised, dried in a hot air oven at 60 C to constant weight and hydrolyzed in 6 N hydrochloric acid for 4 hours at 130 C. The hydrolysates were neutralized to pH 7.0 and subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid and the colour was developed with

the help of Ehrlich reagent at 60 C which was then analyzed at 557nm using a spectrophotometer. The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline[16].

Histopathology of regenerated tissue was performed after complete epithelization. Tissues were excised and stored in formalin. The tissues were sliced to 20 µm thick sections using rotary microtome (WES WOX Model, MT-1090 A) and fixed to the slides. The slides were stained with hematoxylin solution for 1 minute, washed with tap water, stained with eosin solution for 30 seconds, and then washed again with eosin stain. The slides were then subjected to series of ethanol and xylene dehydrations. Cover glasses were mounted on the slides and the samples were observed using compound microscope (Motic) and photographed.

Statistical analysis

All the results are expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way ANOVA followed by Dunnett's *t*-test. Data were considered significant at $P < 0.01$.

Results and discussion

Preliminary phytochemical study

Phytochemical investigation of *Jatropha gossypifolia* root extracts revealed that petroleum ether extract contains phytosterols and fixed oils. Chloroform extract contains alkaloids and phytosterols. Methanol extract revealed the presence of flavonoids, phenolics and tannins. Aqueous extract contains carbohydrates, glycosides, saponins, proteins, amino acids, starch phenolics and tannins. The percentage yield of petroleum ether, chloroform, methanol and aqueous extract were found to be 5.50, 5.81, 7.65 and 12.50, respectively.

Incision wound model

The breaking strength of incision wounds in treated groups increased to significant ($P < 0.01$) extent (Table 1). The results demonstrate the increased tensile strength of wounds in rats and reveal that the methanol extract has impending wound healing activity similar to nitrofurazone ointment as indicated by significant decrease in wound area and epithelisation time. The tensile strengths of wounds treated with ointments containing 5% and 10% w/w methanolic extracts were found to be 695.75 and 759.50 g, respectively, which was more as compared to nitrofurazone ointment treated animals (653.92g).

Table 1 Effect of *Jatropha gossypifolia* root extracts on wound healing of incision model in rats

Sr.No.	Group	Tensile strength	Increase in tensile strength (%)
		(in grams)	
		Statistical mean ± S.E.M.	
1	Control	454.92±4.19	–
2	Standard	653.92±4.43**	43.74
3	Petroleum ether extract (5% w/w)	597.33±3.29**	31.30
4	Petroleum ether extract (10% w/w)	627.75±3.70**	37.99
5	Chloroform extract (5% w/w)	515.75±3.96**	13.37
6	Chloroform extract (10% w/w)	581.83±4.08**	27.90
7	Methanol extract (5% w/w)	695.75±4.15**	52.94
8	Methanol extract (10% w/w)	759.50±3.59**	66.95
9	Aqueous extract (5% w/w)	529.25±3.96**	16.34
10	Aqueous extract (10% w/w)	574.08±3.89**	26.19

Values are expressed as mean ± S.E.M. n= 6. P values were analysed using One-way ANOVA followed by post hoc Dunnett's test.

** $P < 0.01$ versus the control group.

Diabetic excision wound model

Delayed cutaneous wound healing is a chronic problem in diabetic patients and is caused mainly by hyperglycemia, diminished expression of cytokines, oxidative stress, microbial infections and vascular insufficiency. Several other diabetic complications like nephropathy, neuropathy, atherosclerosis and foot deformities add to the severity of the disease and in the development of chronic wounds in diabetic patients which further lead to ulceration, necrosis and amputation[17,18,19]. The measurement of the progress of the wound healing in the diabetic excision wound

model is shown in Table 2. It was observed that the methanol and aqueous extracts in both the concentrations of 5% w/w and 10% w/w significantly ($P < 0.01$) reduced the epithelization time of animals. Aqueous extract (10% w/w) was found to be most potent with activity more than that of the standard treated groups. The hydroxyproline content of chloroform, methanol and aqueous extract treated groups was found to be significantly higher than the standard group ($P < 0.01$).

Table 2 Effect of *Jatropha gossypifolia* root extracts on wound healing of excision model in diabetic rats

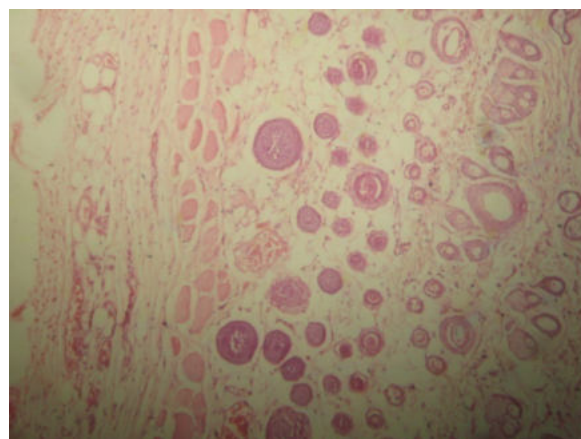
Sr. No	Group	Percentage contraction in wound area					Epithelization period (in days)	Hydroxyproline content (µg/mg of skin)
		3 DAYS	6 DAYS	9 DAYS	12 DAYS	15 DAYS		
1	Control	17.67±1.20	43.67±1.20	64.33±1.30	79.33±1.33	92.67±1.33	16.66±0.42	274.67±6.36
2	Standard	21.67±1.20	45.67±1.20	73.50±0.89	95.67±1.20	100	13.0±0.26**	496.67±7.02**
3	Petroleum ether extract (5% w/w)	17.33±0.99	42.33±0.95	62.33±1.20	75.67±1.20	94.33±1.20	16.83±0.40**	295.33±4.37**
4	Petroleum ether extract (10% w/w)	15.67±0.95	39.33±0.99	56.67±0.99	67.33±0.99	87.17±0.83	17.33±0.21**	323.33±3.53**
5	Chloroform extract (5% w/w)	20.33±1.20	39.67±1.20	62.33±1.20	87.17±0.88	100	14.0±0.37**	522.67±4.37**
6	Chloroform extract (10% w/w)	23.67±1.20	43.33±0.99	68.33±0.95	95.33±1.17	100	13.17±0.31**	556.33±4.91**
7	Methanol extract (5% w/w)	24.33±0.95	48.33±1.20	78.67±0.99	97.33±0.99	100	12.83±0.31**	563.33±4.81**
8	Methanol extract (10% w/w)	26.33±0.95	53.67±1.20	82.67±0.99	100	–	12.17±0.30**	614.67±4.06**
9	Aqueous extract (5% w/w)	27.67±1.20	55.33±1.17	82.67±0.99	100	–	12.33±0.21**	607.33±4.10**
10	Aqueous extract (10% w/w)	29.67±0.95	58.33±0.95	87.67±1.20	100	–	11.33±0.33**	622.33±4.33**

Values are expressed as mean ± S.E.M. n= 6. P values were analysed using One-way ANOVA followed by post hoc Dunnett's test.

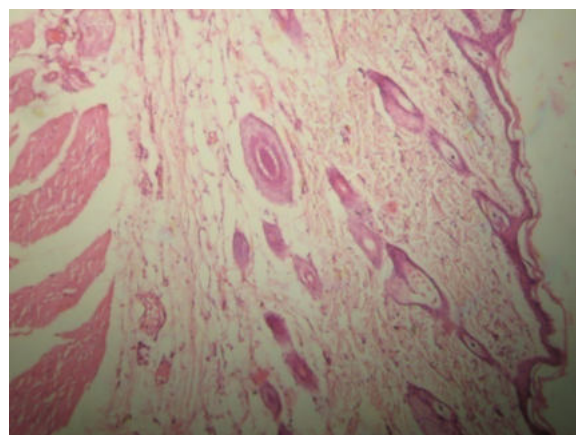
**P< 0.01 versus the control group.

Collagen is the predominant extracellular protein in the granulation tissue of a healing wound and there is a rapid increase in the synthesis of this protein in the wounded area soon after an injury. Measurement of hydroxyproline, which is the product of collagen breakdown, is used as an index of collagen turnover[20]. Histopathological study revealed increased collagen deposition in methanol and aqueous extract treated groups (Figure. 1c and 1d, respectively) analogous to standard group (Figure. 1b) as compared with control group (Figure. 1a).

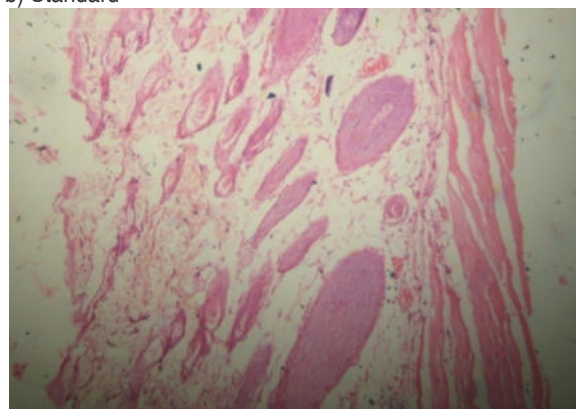
Figure 1 Microphotographs of regenerated skin in excision diabetic wound model



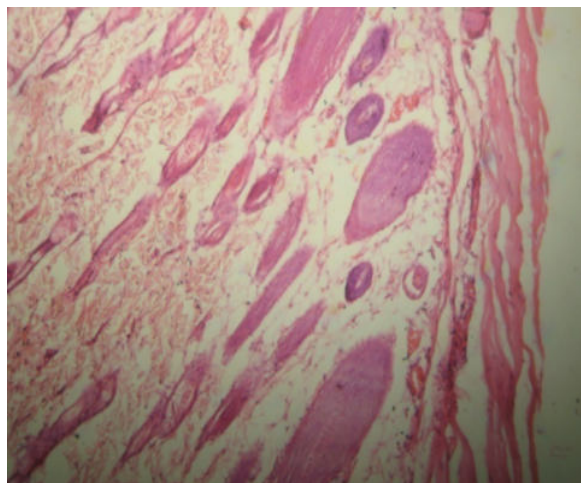
a) Control



b) Standard



c) Methanol extract (10 %w/w) ointment



d) Aqueous extract (10 %w/w) ointment

The results demonstrate potential wound-healing property of the drug by increasing the collagen synthesis, as indicated by elevated hydroxyproline content, probably due to the presence of a mixture of phytoconstituents like alkaloids, glycosides, flavonoids, saponins, phytosterols, fats, proteins, amino acids, phenolics and tannins in the plant.

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Conclusions

The present report is the first comprehensive study to evaluate *in vivo* wound healing potential *Jatropha gossypifolia* roots. The results demonstrate potential wound-healing property of the drug by increasing the collagen synthesis. Further studies may be carried out in order to identify and isolate the bioactive phytoconstituents present in the plant. The study justifies the use of *Jatropha gossypifolia* roots in folklore medicine in India.

Authors' contributions

SKS and HS designed and planned the study. HS carried out the experimental work, biochemical analysis, statistical analysis, and interpretation of results. HS drafted and revised the manuscript. SKS checked and corrected the language. Both authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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