PHARMACOLOGICAL EVALUATION OF *JATROPHA CURCAS* LINN (STEM BARK) FOR WOUND HEALING POTENTIAL IN RATS

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Summary

The methanol: acetone: water (70: 20: 10) stem extract of *Jatropha curcas* L., is used in Traditional African Medicine (TAM) as wound healing agent. However, the scientific basis for this usage has not been established. The present study used to evaluate the wound healing potential of *Jatropha curcas* L. stem bark using pharmacological models. Excision and incision wounds were inflicted upon four groups of six rats each. Group I was assigned as control (ointment base), Group II was treated with standard silver sulfadiazine (0.01%) cream, Group III and Group IV was treated with 5% and 10% extract ointment respectively. The parameters observed were percentage of wound contraction, hydroxyproline content and tensile strength including histopathological studies. It was noted that the effect produced by the extract ointment showed significant healing in both the wound models when compared with control group. All parameters such as wound contraction, hydroxyproline content, tensile strength and histopathological studies showed significant changes when compared to control.

Keywords: Histopathological, Hydroxyproline, Euphorbiaceae, sulfadiazine

Introduction

Herbal medicines have been enjoying revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very crucial and needs imperative attention in order to know the value of the plant. The assessment of the plants for their therapeutic activity is done on the basis of either their chemotaxonomic examination or ethnobotanical information for a particular disease.[1]

Jatropha curcas L. or physic nut, is a bush or small tree (up to 5 m height) and belongs to the Euphorbiaceae family and contains approximately 170 known species.[2] *Jatropha*, a drought-resistant shrub or tree, which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia.[3] It is a multipurpose, drought resistant, perennial plant gaining lot of importance for the production of biodiesel. It has thick glorious branch lets. The tree has a straight trunk and grey or reddish bark, masked by large white patches. It has green leaves with a length and width of 6 to 15 cm, with 5 to 7 shallow lobes. The branches contain whitish latex, which causes brown stains. Inflorescences are formed terminally on branches. The plant is monoecious and flowers are unisexual.[4, 5] After pollination, a trilocular ellipsoidal fruit is formed. The seeds are black and in the average 18 mm long and 10 mm wide ripe Jatropha fruits.[6] It is a multipurpose species with many attributes and considerable potential. The wood and fruit of *Jatropha* can be used for numerous purposes including fuel. It is used against dermatomucosal diseases, arthritis, gout, jaundice, Toothache, gum inflammation, gum bleeding, diarrhoea and pyorrhea**.**[7] Plant extract used to treat Allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies and small pox. Water extract of branches used in HIV, tumor and Wound healing. The plant contains Organic acids, Cyclic triterpenes stigmasterol,[8] Curcacycline A, Curcin,[9] a lectin Phorbolesters Esterases, Sitosterol and its d-glucoside.[10] The leaf and bark have been shown to contain glycosides, tannins, phytosterols, flavanoids and steroidal sapogenins.[7]

The plant is reported to have properties against diseases. In view of these cited activities, observations and traditional uses of plant, the present study was undertaken to explore the wound healing potential of extract of this plant in excision and incision experimental models.

Materials and methods

Plant material

Fresh stem bark of *Jatropha curcas* L. was collected from a local area of Jaipur were identified in the department of botany, Rajasthan University, Jaipur. A voucher specimen number RUBL20844 was deposited in the department. The fresh stem bark was air-dried to constant weight, pulverized and stored in an air-tight container for further use.

Extraction of plant drug

Powder of dried stem bark was subjected to soxhlet extraction with methanol: acetone: water (70: 20: 10). The extract was then filtered and the filtrate was concentrated to dryness.

Preliminary Phytochemical Screening

The extract was subjected to phytochemical tests for tannins, steroids, alkaloids and glycosides, flavanoids, carbohydrates, proteins and amino acid using reported methods.[11, 12]

Preparation of formulation and standard used

5% (w/w) & 10% (w/w) simple ointment containing the extract of plant was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin base. For this, 5 g & 10 g extract was incorporated in 100 g of the base. Silver sulfadiazine (0.01%) was used as standard drug for comparing the wound healing potential of extract in different animal models.

Animals

Albino rats of either sex (150-200 g) were used for experimental purpose. The animals were housed in hygienic cages (6 rats / cage) under standard conditions of temperature $(25\pm2)^0$C, relative humidity (45±20) % and (light) 12h: (dark) 12h cycle. The rats were fed with standard pellet diet (Amrut feeds, Chakan) and water *ad libitum*. The animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days prior to the experiments. The experimental design and research plan along with animals handling and disposal procedure were approved by Institutional Animal Ethical Committee of Jaipur National University (1054/ac/07/CPCSEA) and IAEC approval number was JNU/IAEC/2010/02.

Grouping of animals

Four groups of animals containing six in each were used for excision and incision wound models. The animals of groups I, II and III, 1V were considered as the control, reference standard and treated (5%) & (10%) respectively.

In vivo studies

Excision wound model

The animals were divided into four groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 500mm^2 full thickness of skin from a predetermined shaved area.[13] The wounds were left undressed to the open environment. The ointment base, standard drug ointment (0.1% silver sulfadiazine) and extract of plant ointment (5%, w/w) & (10%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation.[14, 15]

Incision wound model

In incision wound model,[16] all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.[17]

Wound healing evaluation parameters

Measurement of wound contraction

An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated.[18] The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 500mm^2, as 100%, by using the following formula as

% wound contraction

= initial wound size − specific day wound size÷ initial wound size× 100

Measurement of tensile strength

The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer.[19] In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Hydroxyproline estimation

Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70 ˚C to constant weight and were hydrolysed in 6NHCl at 130◦C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4M perchloric acid and colour was developed with the help of Ehrlich reagent at 60 ◦C. The absorbance was measured at 557nm using a spectrophotometer. The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline.[20]

Histopathological examinations

A specimen sample of skin tissues from control, standard and treated group was taken out from the healed wounds of the animals in incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin[21] and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation, and angiogenesis.

Statistical analysis

Results obtained from both wound models have been expressed as mean±SEM and the treated group was compared with control group. The results were analyzed statistically using Dunnet test followed by one-way ANOVA, to analyze the differences between the treated and control. The data were considered significant at $P < 0.01$.

Results

Preliminary Phytochemical Screening

The Preliminary phytochemical investigation revealed the presence of various phytoconstituents in *Jatropha curcas* Stem Bark extract. It showed the presence of phytoconstituents and their results are given in Table 1.

Table 1: Preliminary qualitative tests of *Jatropha curcas* Stem bark extracts

S.NO.	TESTS	JCMSE
1.	Alkaloids	++
2.	Glycosides	++
3.	Carbohydrates	++
4.	Flavanoids	++
5.	Triterpenoids	++
6.	Saponin	++
7.	Tannin and Phenolic compound	++
8.	Protein and Amino acid	++
9.	Steroid	++
10.	Fixed Oil & Fat	-

[+] – Present, [-] – Absent

Wound contraction

A better healing pattern with complete wound closure was observed in standard and treated group (5%, w/w) ,(10%, w/w) within 17, 22 & 19 days respectively while it was about 27 days in control rats as shown in Table 2 and Graph 1.

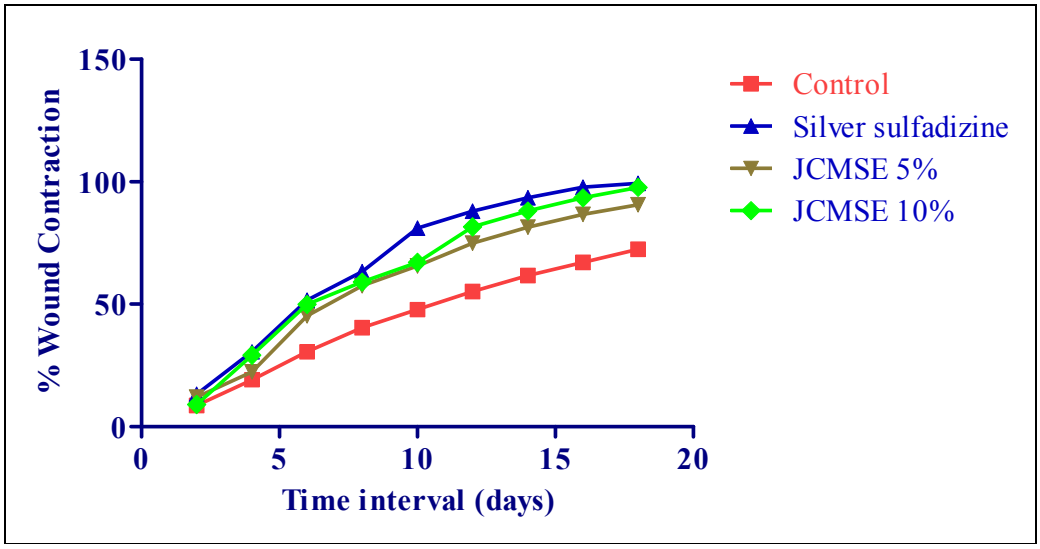
Table 2: Effect of JCMSE and standard ointment on % wound contraction of excision wound models in rats

	% wound contraction			
Post wounding Days	control	Standard	JCMSE ointment (5%)	JCMSE ointment (10%)
2	8.72 ±1.791%	13.31 ±1.229%	12.11 ±1.538%	9.03 ±3.02%
4	19.13 ±1.528%	30.59 ±2.492%*	22.45 ±1.748%**	29.14 ±2.688%
6	30.55 ±3.055%	51.74 ±0.564%***	45.34 ±3.332%***	49.95 ±2.164%***
8	40.47 ±2.107%	63.32 ±2.538%***	57.56 ±3.396%***	59.0 5±2.816%***
10	47.79 ±1.51%	81.04 ±3.016%***	65.65 ±2.068%***	67.11 ±2.729%***
12	55.21 ±2.473%	87.90 ±2.488%***	74.98 ±1.469%***	81.56 ±2.791%***
14	61.70 ±2.91%	93.49 ±1.412%***	81.38 ±1.790%***	88.11 ±2.049%**
16	67.12 ±3.276%	97.82 ±0.311%***	86.64 ±1.331%***	93.39 ±0.723%***
18	72.41 ±3.602%	99.29 ±0.113%***	90.54 ±1.086%***	97.63 ±0.345%***
Epithelization period (days)	27.17 ±0.454	17.76 ±0.398***	22.44 ±0.617***	19.35 ±0.398***

Values are expressed as mean ± S.E.M. (n= 6). * P< 0.05, ** P< 0.01, *** P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Graph 1

% Wound contraction of excision wound models in rats



Tensile strength of incision wound model

Tensile strength for the treated group on 10th day was found to be significant ($P < 0.001$) than control group as shown in Table 3 and Figs. 1 and 2.

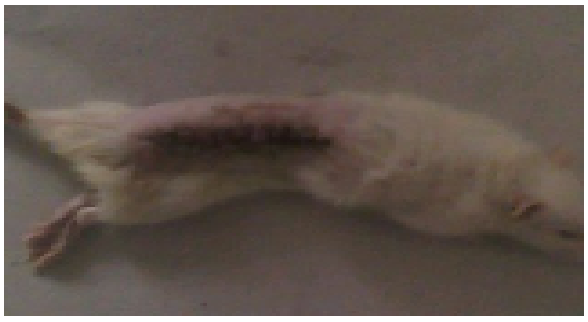


Fig. 1. Stitched incision wound with black silk thread during incision model

Fig. 2. Breaking strength measurement in incision wound model

Hydroxyproline content

Treated group showed significant increase in hydroxyproline content when compared to control group ($P < 0.001$) as depicted in Table 3.

Table 3

Effect of JCMSE and standard ointment on various wound parameters of incision wound model in rats

Groups	Hydroxyproline (mg/g tissue)	Tensile strength (g/mm^2)
Control	25.76±0.003	413.80±3.665
Standard	61.52±0.004***	607.22±3.717***
JCMSE ointment (5%)	43.39±0.002***	493.75±4.136***
JCMSE ointment (10%)	55.76±0.003***	582.80±3.665***

Values are expressed as mean ± S.E.M. (n= 6). *** P<0.001 as compared to control. One way Anova followed by Dunnett's multiple comparison test.

Histopathological examinations

In standard and treated albino rats with extract (5%) & (10%), excision and incision type of wounds have shown significant healing as in fibroblasts cells (F), collagen fibres (CF) and new blood vesicles (BV) in Figs. A, B and C respectively. While in control rats wounds showed

incomplete healing in Fig. D. Control group has shown to slightest wound healing ability when compared to extract treated and reference ointment group. Fibroblast cells, collagen fibres and blood vessels are prominently present in standard and extract treated group as compared to control.

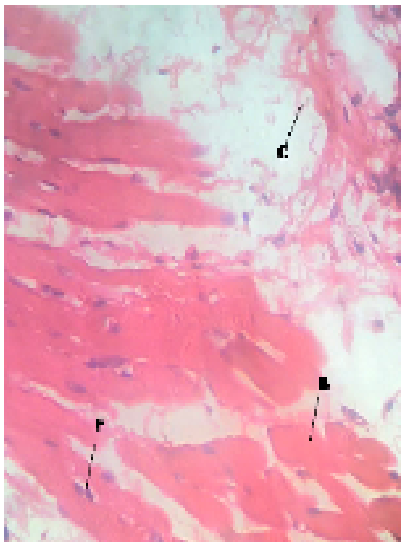


Fig. A

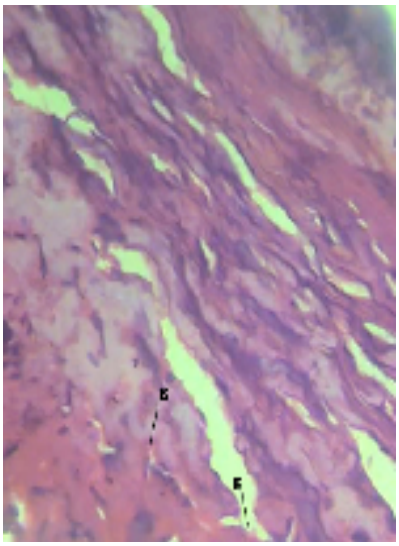


Fig. B

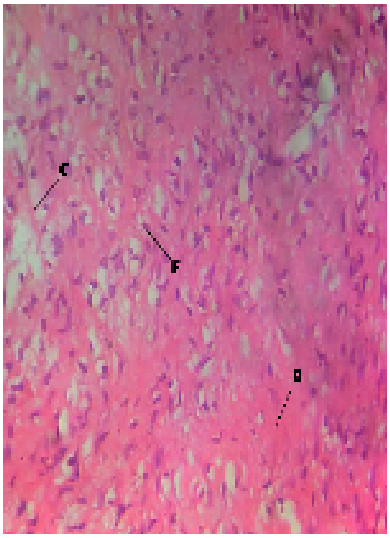


Fig. C

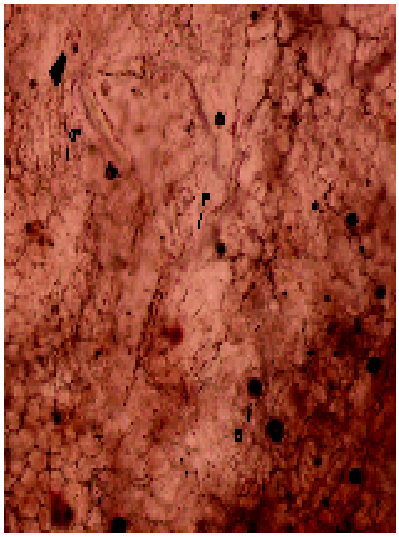


Fig. D

Fig. 3. In standard and treated albino rats with extract (5%) & (10%), excision and incision type of wounds has showed significant healing as in fibroblasts cells (F), collagen fibres (CF) and

new blood vesicles (BV) in Figs. A, B, C respectively. while in control rats wounds shown incomplete healing in Fig. D.

Discussion and conclusion

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair.[22] Hence in this study, excision and incision wound models were used to evaluate the effect of extract ointment on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres.[23] Increase in blood vessels and role of antioxidants were experimentally proved.[24] In excision wound, the extract showed faster healing with earlier wound contraction compared with control groups. The earlier wound contraction rate of the extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue.[25] The extract of plant increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure, it demonstrates remarkable visco-elastic and hygroscopic properties which are relevant for dermal tissue function. Biological activities in skin are due to its interaction with various binding proteins. Due to an influence on signaling pathways, hyaluronic acid and hydroxyproline is involved in the wound-healing process and scarless fetal healing. In clinical trials, topical application of hyaluronic acid has improved the healing of wound.[26] In addition, the muco-polysaccharide hyaluronic acid protects granulation tissue from oxygen free radical damage and thereby stimulates wound healing.[27] Among the glycosaminoglycans, hydroxyproline, dermatan sulfate and dermatan have also been implicated in wound repair and fibrosis. Their ability to bind and alter protein–protein interactions has identified them as important determinants of cellular responsiveness in development, homeostasis and disease.[28]

The results showed that extract ointment possesses a distinct prohealing stroke. This was demonstrated by a significant increase in the rate of wound contraction. Significant increase ($P < 0.01$) in tensile strength, and hydroxyproline content were observed, which was auxiliary supported by histopathological studies. This indicated newly formed fibroblasts cells, collagen fibres and blood vessels. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids, triterpenoids and tannins are known to promote the wound-healing process.[29, 30, 31]

Preliminary phytochemical screening of extract of *Jatropha* showed the presence of alkaloids, flavonoids and tannins. Its chemical constituents mainly consist of oils and fats, org. acids, flavonoids, triterpenes, steroids, sterols, and proteins. The wound healing action of *Jatropha* may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents.

Hence, the results obtained from data concludes that extract ointment of plant has properties that render it capable of promoting wound healing activities such as stimulating wound contraction and increasing tensile strength of incision as compared to control.

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References

1. Juneja JD. Shrivastava PN, Guha MK, et al. Preliminary phytochemical screening of some folklore medicinal plants for their anti-inflammatory activity. Pharmacognosy Magazine 2007; 11: 201–203.
2. Linnaeus C. Species plantarum in *Jatropha*. Impensis Laurentii Salvii Stockholm 1753; 1006–1007.
3. Heller J. Physic nut, *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops, Institute of Plant Genetics and Crop Plant Research. Gartersleben, International Plant Genetic Resources Institute, Rome 1996; 1.
4. Gupta RC. Pharmacognostic studies on ‘Dravanti’ Part I *Jatropha curcas* L. Proc Indian Academic Science (Plant Science) 1985; 94: 65–82.

5. Dehgan B, Webster GL. Morphology and Intrageneric Relationships of the Genus *Jatropha* (Euphorbiaceae). University of California Publications in Botany. 1979; 74.
6. Singh RP. Structure and development of seeds in Euphorbiaceae, *Jatropha* species. Beitr Biol Pflanz 1970; 47: 79–90.
7. Okoli CO. Akah PA, Ezike AC, et al. Ethnobiology and pharmacology of *Jatropha curcas* L. Ethnopharmacology 2008; 101-125.
8. Khafagy SM. Mohamed YA, Abdel NA, et al. Phytochemical study of *Jatropha curcas*. Plant Med 1977; 31: 274–277.
9. Nath LK, Dutta SK. Acute toxicity studies and wound healing response of curcain, a proteolytic enzyme extract from the latex of *Jatropha curcas* L. In: Gubitz GM, Mittelbach M, Trabi M, eds. Biofuels and Industrial Products from *Jatropha curcas*, DBV Graz, 1997: 82-86.
10. Naengchomnong W. Thebtaranonth Y, Wiriyachitra P, et al. Isolation and structure determination of four novel diterpenes of *Jatropha curcas*. Tetrahed Lett 1986; 27: 2439–2442.
11. Khandelwal KR. Practical Pharmacognosy. 9th ed. Pune, Nirali Prakashan, 2002.
12. Ansari SH. Essential of Pharmacognosy. 1st ed. New Delhi, Birla publications Pvt Ltd, 2005-06.
13. Saha K. Mukherjee PK, Das J, et al. Wound healing activity of *Leucas lavandulaefolia* Rees. Journal of Ethnopharmacology 1997; 56: 139–144.
14. Taranalli AD. Tipare SV, Kumar S, et al. Wound healing activity of *Oxalis corniculata* whole plant extract in rats. Indian Journal of Pharmaceutical Sciences 2004; 66: 444–446.
15. Anderson JE. Muirs Text Book of Pathology. 11th ed. ELBS, 1980; 77–85.
16. Ehrlich HP, Hunt TK. The effect of cortisone and anabolic steroids on the tensile strength of healing wounds. Annal Surgery 1968; 57: 117.
17. Hemalata S. Subramanian N, Ravichandran V, et al. Wound healing activity of *Indigofera ennaphylla* Linn. Indian Journal of Pharmaceutical Sciences 2001; 63: 331–333.
18. Sadaf F. Saleem R, Ahmed M, et al. Healing potential of cream containing extract of *Sphaeranthus indicus* on dermal wounds in Guinea pigs. Journal of Ethnopharmacology 2006; 107: 161–163.

19. Kuwano H. Yano K, Ohano S, et al. Dipyridamole inhibits early wound healing in rat skin incisions. Journal of Surgical Research 1994; 56: 267–270.
20. Woessner JF. The determination of hydroxyproline in tissue and proteinsamples containing small proportions of this imino acid. Arch Biochem Biophys 1961; 93: 440–447.
21. McManus JFA, Mowry RW. Staining Methods, Histological and Histochemical. Harper & Row/Evanston, New York/London 1965.
22. Charles VM, Rusell RCG, Williams NS. Short Practice of Surgery. 20th ed. London, Champan and Hall, 1995.
23. Udupa AL, Kulkarni DR, Udupa SL. Effect of *Tridax procumbans* extracts on wound healing. International Journal of Pharmacognosy 1995; 33: 37–40.
24. Michel JW. Wound healing-oxygen free radicals and wound healing. Clinics in Plastic Surgery 1990; 17: 1473–1483.
25. Moyer KE, Saggers GC, Ehrlich HP. Effects of interleukin-8 on granulation tissue maturation. Journal of Cellular Physiology 2002; 193: 173–179.
26. Weindl G, Schaller M, Korting HC. Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin Pharmacology and Physiology 2004; 17: 207–213.
27. Bayliss MT. Proteoglycans: structure and molecular organisation in cartilage. In: Hukins DWL, eds. Connective Tissue Matrix, London, Macmillan, 1984: 55.
28. Trowbridge JM, Gallo RL. Dermatan sulphate-new functions from an old glycosaminoglycan. Glycobiology 2002; 12: 117–125.
29. Tsuchiya H. Sato M, Miyazaki T, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of Ethnopharmacology 1996; 50: 27–34.
30. Scortichini M, Pia RMJ. Applied Bacteriology. 1991; 71: 109.
31. Rane M., Madhura MA., Kumari S. Comparative effect of oral administration and topical application of alcoholic extract of *Terminalia arjuna* bark on incision and excision wounds in rats. Fitoterapia 2003; 74: 553– 558.