

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-1: Jan-Mar -2011

IIIARPT ISSN 0976-4550

WOUND HEALING ACTIVITIES OF EUGENIA JAMBOLANA LAM. BARK **EXTRACTS IN ALBINO RATS**

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ABSTRACT: Wound healing is physiological process, which takes place by body's natural regenerative capacity. Due to various reasons there may be delay in healing and this prolonged healing may sometimes lead to scar formation. Currently attention has been focused on natural products to prevent infection and to promote healing. In the present study, Eugenia jambolana bark extracts was taken to investigate its wound healing property. Full thickness deep burn wound model in Albino rats, were used to study the healing efficiency. Formulations (10% ointment) of crude ethanolic extract of the Eugenia jambolana bark was applied tropically over thermal wound. It was found that ointment treated rats showed accelerated healing than the control. It was observed that 10% extract of the Eugenia jambolana bark has progressive effects on wound healing in the experimental groups. This study suggests that *Eugenia jambolana* bark powder could be developed as a therapeutic agent for wound healing.

Keywords: Eugenia jambolana, wound healing activity

INTRODUCTION

Wound healing is a complication interaction of many factors. This phenomenon has mystified early and modern man. This is evident from Archeological findings which showed that ancient man also had to use a variety of tools to deal with various injuries inflicted on him under hard conditions and during wars (Manjo, 1991). The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases (Cragg et al., 1997). Eugenia jambolana Lam. (Syn. Syzygium cumini (L) Skeels or Syzynium jambolana DC.) belonging to the family Myrtaceae is a large evergreen tree up to 30 m high. Bark pale brown, slightly rough on old stems. Fruit is one seeded berry and blue. It is widely distributed through out India, Ceylon-Malaya and Australia and known as Jamun, Jam, Jambul in India. It has been valued in Ayurveda and Unani systems of medicine for possessing variety of therapeutic properties. Most of the plant parts of E. jambolana are used in traditional systems of medicine in India. According to Avurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers (Kirtikar and Basu, 1975). It is also acts as a gargle in sore throat; spongy gums etc. and when externally used, bark shows good wound healing properties (Priyavtra Sharma and Mehta, 1969; Nadkarni, 1954). This study was designed to explore the healing effects of topically applied ointment prepared from *Eugenia jambolana* leaves extracts in rat intraoral wound.

MATERIALS AND METHODS

Collection of plant materials

Barks of Eugenia jambolana Lam .were collected in Perambular district, Tamil Nadu during the month of January 2005. The collected plant were botanically identified and dried at room temperature.

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Experimental Animals

The protocol of the study was approved by the Local Ethical Committee for animal experimentation. Healthy adult Wistar albino rats (150-180g) were obtained from Venkateshwara Enterprises, Bangalore and used in wound healing model experiments. The rats were divided into four groups of six animals each. The rats were used after an acclimatization period of 7 days to the laboratory environment. Animals were housed in metal cages and provided with standard food and tap water ad libitum during the whole period of the experiment. The changes were observed on 10^{th} and 16^{th} day.

Preparation of plant extract

The collected plant parts were pulverized by a mechanical grinder, sieved through 40 mesh. The powdered materials were extracted with ethanol using soxhlet extraction apparatus. This ethanolic extract was then concentrated and dried under reduced pressure. The ethanol free semi-solid mass thus obtained was used for the experiment.

Ointment Formulation

Two types of ointment formulation were prepared from the extract 5% (w/w), 10% (w/w), where 5 g and 10 g of the extract were incorporated in 100 g of simple ointment base B.P. respectively. Nitrofurazone ointment (0.2% w/w, Smithkline – Beecham) was used as a standard drug for comparing the wound healing potential of the extract of the bark.

Incision wound

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm full thickness of skin from the depilated area; the wound was left undressed to the open environment. No medication was used throughout the experiment. After the incision was made, the ointment was applied to the wound once a daily in the experimental group animals. The progressive changes in wound area were measured in mm at every 3 days interval. Progressive decrease in the wound size was monitored periodically.

Qualitative Phytochemical Evaluation

The methanolic extract of the bark was subjected to qualitative tests for the identification of the phytoconstituents present in it *viz.* alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats, phenolic compounds and tannins, proteins and free amino acids, gums and mucilage, flavanoids, lignin and saponins.

RESULTS AND DISCUSSION

In the preliminary phytochemical evaluations of the ethnolic extract of *Eugenia jambolana* Lam. bark powder showed the presence of alkaloids, phytosterol, phenolic compounds and tannins, flavanoids and lignin.

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2 w/w), 5% w/w extract ointment, 10% w/w extract ointment and the control group were reported in the Table: 1. It is observed that the wound contracting ability of the extract ointments were significantly greater than that of the control, which was comparable to that of the reference standard, NFZ ointment. A better healing pattern with complete wound closure was observed in rat at 10^{th} day and 16^{th} day by 5% w/w and 10% w/w extract ointments respectively.

The process of wound healing occurs in four phases: (i) Coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition, any agent which accelerates the above process is a promoter of wound healing. Plant produces have been showed to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing, due to the presence of active terpenes, alkaloids and flavanoids (Suguna *et al.*, 1996). A glycosidal mixture extract of *Centella asistica* has been reported to be responsible for enhanced repair only in incised wounds (Rosen *et al.*, 1967) and in stimulating collagen in human skin fibroblast cells (Vogel and De Souza, 1991).



Tannins and anthraquinones are the major phytoconstituent present in this plant which may be responsible for wound healing action. The plant *Portulaca oleracea* containing the tannins possesses wound healing activity as that of the *E. jambolana* (Rashed, 2003). The gel of ethanolic extract of the plant *Vernonia scorpioides* possess wound healing action by improving regeneration and organization of the new tissue due to the presence of tannins (Leite, 2002). The wound healing property of the extract of the *Eugenia jambolana* bark appears to be due to the presence of its active principle which accelerative the healing process and confers breaking strength to the healed wound.

Conclusion

Wounds one visible results of individual cell death or damage, and can be classified by site, size, depth and causation – surgery, accident or circulatory failure etc. The present study was undertaken to evaluate the wound healing activity of the extract of the *Eugenia jambolana* bark in excision. In excision wound model the extract ointment showed faster epithelialization, when compared with the control. Further investigations are necessary to determine the bioactive constituents present in the extracts used for studies.

Table: 1 The preliminary phytochemical screening of the methanolic extracts of Eugenia jambolana Lam.

Phytoconstutient	Result			
Alkaloids	+			
Reducing Sugar	-			
Phytosterol	+			
Fixed oil & Fats	-			
Phenolic compounds & Tannins	+			
Proteins & Amino Acids	-			
Gums & Mucilage	-			
Flavonoids	+			
Lignin	+			
Saponins	-			
* + = Present, $- =$ Absent				

Table – 2 Effect of extract of *Eugenia jambolana* Lam. bark and Nitrofurazone on excision wound model

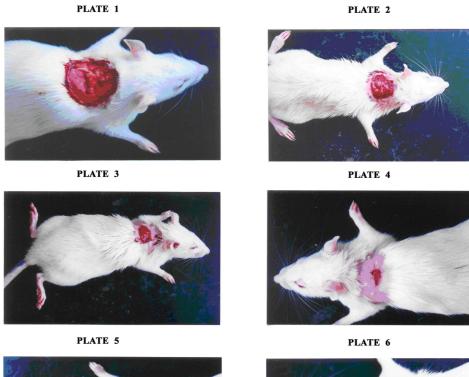
Post	Wound Area (mm²)			
wounding days	Simp le ointment (Contro l)	Nitrofurazone ointment (0.2% w/w)	Extract ointment (5% w/w each)	Extract comb ination (10% w/w each)
0	530±33.6(0)	516±36.8(0)	514±21.0(0)	507±39.8(0)
2	509±18.6(3.9)	458±36.8(11.2)	372±18.8(27)	338±14.8(33)
4	465±13.8(12.2)	318±12.6*(38.3)	3 12±19.9 *(39)	268±18.6*(47)
6	424±30.1(20.0)	270±14.7*(47.6)	245±15.3 *(52)	171±19.4**(66)
8	389±14.8(26.6)	193±11.4**(62.5)	162±12.5**(68)	105±9.8**(79)
10	345±23.6(34.9)	110±8.6**(77.3)	95±9.6**(81)	68±5.9**(86)
12	269±14.3(49.2)	79±6.3 **(84.6)	66±7.4**(87)	36±2.1**(92)
14	215±11.3(59.4)	36±1.6 **(93.0)	37±3.5**(92)	14±1.1**(97)
16	189±14.3(64.3)	10±1.9 **(98.0)	19±0.8**(96)	0.0**(100)
18	171±15.1(67.7)	0.0**(100)	0.0**(100)	0.0**(100)

Values are mean \pm S.E of 6 animals in each group. Figures in parenthesis indicate percentage of wound contraction. *p<0.01., **p<0.001 vs. respective control by students t-test

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ISSN 0976-4550







- PLATE 1: CONTROL WOUND WITH NITROFURAZONE OINMENT (initial day)
- PLATE 2: CONTROL WOUND WITH NITROFURAZONE OINMENT (day 10)
- PLATE 3: CONTROL WOUND WITH NITROFURAZONE OINMENT (day 16)
- PLATE 4: CONTROL WOUND WITH NITROFURAZONE OINMENT (day 26)
- PLATE 5: EXPERIMENTAL TREATED WITH EUGENIA JAMBOLANA BARK EXTRACT 10% W/W (day 10)
- PLATE 6: EXPERIMENTAL TREATED WITH EUGENIA JAMBOLANA BARK EXTRACT 10% W/W (day 16)

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