Research Article

EFFECT OF OIL EXTRACT OF *FICUS RELIGIOSA* BARK ON WOUND HEALING ACTIVITY IN ALBINO RATS

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ABSTRACT

Bark of *Ficus religiosa(FR)* is used traditionally for the treatment of variety of wounds. In this study oil extract of *FR* stem bark has been used to evaluate the effect of it on incision and excision wound models in rats. Four groups of animals, [control- (distilled water), vehicle control – (coconut oil), standard – (aloe vera), test – (oil extract of *FR*)] were used to study the wound breaking strength in incision wounds and percentage of wound contraction and period of epithelisation in excision wounds. Topical application of the oil extract of *FR* has shown significant increase in the wound breaking strength ($p<0.01$) in incision wound model; reduction in period of epithelisation and increase in wound contraction rate ($p<0.001$) were observed in excision wound model when compared to the control. This study ascertains the wound healing activity of *FR* and substantiates the use of *FR* bark in ayurveda for treatment of wounds.

Keywords: Wound contraction, Incision wound model, Excision wound model, Period of epithelisation, *Ficus religiosa.*

INTRODUCTION

Wound is a breach in the normal tissue continuum that results in a variety of cellular and molecular sequelae. Healing is required for normal functioning of the tissue. Various factors can enhance normal wound healing[1] or reverse depressed wound healing due to drugs like corticosteroids[2], anticancer[3] and non steroidal anti-inflammatory drugs[4] Medicinal plants have been used as an alternative remedy for treating human diseases for centuries because they contain numerous active constituents of therapeutic value[5]. *FR* is commonly referred as the pipal in Hindi and asvattha in Sanskrit.[6] In ayurveda the leaves of this plant are used for the treatment of ulcers, bark is used for the treatment of ulcers, gonorrhoea and diabetes. The roots are used in the treatment mouth ulcer, diarrhoea and dysentery. It has been found that topical application of hydro alcoholic extracts of *FR* leaves promotes wound healing activity in incision, excision and burn wound models in rats[7]. The aqueous and ethanolic extracts of the plant was proved to have antimicrobial activity[8].

In ayurveda practice, oils are used as one of the mode of application in treatment of the wounds. But there is no scientific data available on the wound healing activity of oil extracts of *FR* which is prepared according to traditional methods. Hence, this study was undertaken to evaluate the effect of topical application of oil extract of stem bark of *FR* on incision and excision wound models in rats.

MATERIALS AND METHODS

Experimental animals

Healthy albino Wistar rats of either sex, weighing between 150-200 gms, were used. The experimental protocol was approved by Institutional Animal Ethics Committee. The animals were maintained under standard environmental condition of temperature 23±2°C, humidity 50 ± 5% and 10 -14 hours light and dark cycle in an animal house (CPCSEA). The animals were provided with standard rat feed and water; ad libitum. The animals were housed individually in polyprophylene cages containing sterile husk as bedding after making wounds till the completion of wound healing process.

Study Design

8 groups of animals, containing 6 rats in each group were used for incision and excision wound models.

Group I, V– Control – Distilled water

Group II, VI- Vehicle Control (coconut oil, topical)

Group III, VII- Standard (aloe vera cream, topical) [9]

Group IV, VIII - Test (oil extract of *F.R*, topical)

Preparation of oil extract of *FR*[10]

Bark of plant was collected in the month of December and dried under shade for 15 days. One part of coarse powder of stem bark was added to 16 parts of water and was continued to boil to reduce the volume to one forth. The decoction was strained using a muslin cloth. Coconut oil was taken in a vessel and heated for some time. Then one part fine paste of stem bark was added to 4 parts of coconut oil and 16 parts of above prepared decoction from bark powder. This mixture was boiled on mild fire with stirring to avoid paste to adhere to the vessel and boiling continued till all the water evaporates. Well-cooked oil should not have any residual moisture. The oil was strained while warm through muslin cloth and allowed to cool. Detailed medicated oil processing is

described in ayurvedic textbooks recognized by Drugs and Cosmetic Act[11] and in the Ayurvedic Formulary of India (AFI).[12]

Wound Models

Incision wound model

After 12 hr of fasting, animals were anaesthetized by using injection Ketamine (50mg/ kg body wt i.m.)[13]. Two para vertebral straight incisions of 6 cm were made through the entire thickness of the skin on each side of vertebral column,[2] with the help of a sharp blade. After mopping wounds dry, wounds were closed by using 4-0 silk thread and straight round body needle. The interrupted sutures were placed at equidistance points of 1 cm each. Wounds were then mopped with cotton swabs soaked in 70% alcohol. The animals were housed individually. Drugs were applied once daily from the day of wounding till 9th post wounding day. Removal of the sutures done on 7th post wounding day. The tensile strength was determined on 10th post wounding day by continuous water flow technique.[4] (table 1).

Excision wound model[14]

After fasting for 12 hr, rats were anaesthetized by using injection Ketamine (50mg/kg i.m.). A round seal of 2.5cm diameter was impressed on the dorsal of thoracic region 5 cm away from the ears on shaved back of the rat. Full thickness skin from demarked area was excised to get a wound measuring 500mm^2. After achieving full haemostasis by mopping the wound with cotton swab soaked in warm saline, animals were placed in their individual cages. Animals were treated daily from the day of wounding till 21st post wounding day. In this wound model, two physical attributes of healing namely wound contraction rate and epithelisation period were studied. To monitor wound contraction, the progressive changes in wound area will be followed planimetrically. Wound area was traced on a transparent paper on the day of wounding, 4th, 8th, 12th and 16th days. The tracing were then transferred to 1mm graph sheets to calculate the wound area. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound 500mm^2 as 100% by using the following equation;

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound Size}} \times 100$$

Epithelisation period was monitored by noting the number of days required for the Eschar to fall off leaving no raw wound behind. (table 2).

Statistical Analysis

The results were expressed as Mean ± SEM. Differences between the experimental groups were compared using one-way ANOVA test followed by Bonfererroni's post hoc test.

$p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

In incision wound model, topical application of oil extract of *FR* showed significant increase in the wound breaking strength when compared to control ($p < 0.01$) and vehicle control group ($p < 0.05$). But there was no statistical significance between standard and the test groups.

Table 1: Mean wound breaking strength for incision wounds

Group	Wound breaking strength (gms) (Mean ± SEM)
Control	172 ± 13.15
Vehicle control	183.23 ±6.94
Standard	258.35± 20.75**
Oil extract of *FR*	256.83 ± 18.97**

Normal control vs test ** p< 0.01, Vehicle control vs test *p<0.05

Table 2: Mean wound contraction rate in excision wound

Groups	Percentage of wound contraction (days)			
	4th	8th	12th	16th
Control	13.72 ±2.7	5 8.96 ±2.6	81.66 ± 1.6	91.33 ±0.5
Vehicle Control	18.88 ± 2.6	7 0.36 ± 3.1	8 1.55 ± 1.6	93. 55 ± 0.8
Standard	2 2.36±3.7	75.28± 1.6	88.23 ± 1.5	98.4 ±0.2***
Oil extract of *FR*	18.40 ± 5.3	70.52 ± 1.9*	88.23 ± 1.7*	97.16 ± 0.3***

*p<0.05, test vs control on 8th day and 12th day, ***p<0.001, test vs control on 16th day. Vehicle control vs test *p<0.05 on 8th, 12th and 16th days.

In excision wound model topical application of oil extract of *FR* treated animals showed significant reduction in wound size was on 8th day ($p<0.05$), on 12th ($p<0.05$) and 16th day ($p<0.001$) when compared to control and vehicle control. But there was no significant difference between test and standard.

Topical application of oil extract reduced the period of epithelisation when compared to control group (Table 3).

The results of the present study shows that the topical application of oil extract of *FR* promoted the wound breaking strength, wound contraction and period of epithelization respectively in incision and excision wounds.

In ayurveda system of medicine different types of medicated oils are prescribed to treat specific indications. Medicated oils have principally three components namely, a liquid which may be aqueous decoction of one or more herbs, a fine paste of the herbs and a vegetable oil[15]. Normally crude sesame oil is used though occasionally castor oil and coconut oil is also used, either in parts.

In the present study coconut oil used as vehicle because it is also used in treating wounds traditionally. The present study shows better results in medicated oil treated group than coconut oil treated group and there was no statistical significance between standard and the test groups.

The oil preparation by using above mentioned procedure is economical. It is mentioned in ayurveda books that properly prepared oils have long shelf life of about one year without any separate preservatives[16].

Wound healing is a process by which the damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. Phases of wound healing are collagenation, wound contraction and epithelisation. Intervention into any one of these phases by drugs could either promote or depress one or all phases of healing. Collagen a protein, provides structural support and it is a main component of extracellular tissue[17]**.** Collagen is composed of amino acid, hydroxyproline. The estimated increase in hydroxyproline content of the granulation tissue indicated rapid collagen turnover thus, leading to rapid healing of wounds[18].

Table 3: Period of epithelisation for excision wounds

Groups	Period of epithelisation (days)
Control	19.33 ± 0.494
Vehicle control	19 ± 0.365
Standard	15 ± 1.095
Oil extract of *FR*	14.33±0.802***

The above mentioned prohealing actions of the oil extract of *FR* may be due to the presence of phytoconstituents of phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryl-d-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one[19]**.** The flavonoids[20] and triterpenoids[21] are known to promote the wound-healing process mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and faster epithelisation.

CONCLUSION

This study ascertains that oil extract of *Ficus religiosa* possess good wound healing activity and it substantiate the use of *Ficus religiosa* bark in folklore medicine for treatment of wounds. Further phytochemical studies are suggested to isolate, characterize and identify the specific active compounds in this plant responsible for wound healing activity.

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