

Wound healing activity of the hydro alcoholic extract of *Ficus religiosa* leaves in rats

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Citation

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Abstract

The leaves of *Ficus religiosa* are used traditionally in the folklore for the treatment of various kinds of wounds. The present study was undertaken to verify the effect of hydro alcoholic extract of *Ficus religiosa* leaves on experimentally induced wounds in rats in excision wound, incision wound and burn wound models. A formulation of leaves extract was prepared in emulsifying ointment at a concentration of 5% & 10% and applied to the wounds. In the excision wound and the burn wound models the extract showed significant decrease in the period of epithelization and in wound contraction (50%). A significant increase in the breaking strength was observed in the incision wound model when compared to the control. The result suggests that leaf extract of *Ficus religiosa* (both 5% and 10%) applied topically possess dose dependent wound healing activity.

INTRODUCTION

Wounds are unavoidable events of life; wounds may arise due to any agent that induces stress & injury and their healing has been one of the well-known problems. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Treatment is therefore aimed at minimizing the undesired consequences. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified (1). Hence, there is dearth of rational pro-healing agents for the wound management programme, which can hasten the healing process.

The plant, *Ficus religiosa* (F Moraceae) is grown throughout India and is worshiped by followers of many religions for its contributory nature towards health and well being of mankind. It is commonly referred as the pipal in Hindi and asvattha in Sanskrit (2). In ayurveda the leaves are used for the treatment of ulcers and wounds. The bark is used for the treatment of various skin diseases, scabies, in ulcers, as

astringent and as tonic. The fruits and tender buds are used as laxatives. The juice of its leaves extracted by holding them near the fire can be used as the eardrop. Its power bark has been used to heal the wounds for years. The bark of the tree is useful in inflammations and glandular swelling of the neck. Its root bark is useful for stomatitis, clean ulcers, and promotes granulations. Its roots are also good for gout. The roots are even chewed to prevent gum diseases (3). It has been reported that the leaves contain campesterol, stigmasterol and β & γ Amyrins, tannins, amino acids, piperine, piperlongumine, dihydrolongumine and methyl piperate (4).

Even though, traditionally, leaves of *Ficus religiosa* were extensively used for the treatment of variety of wounds; however, no scientific data in its support is available. The present study was undertaken to ascertain the effect of hydroalcoholic extract of *Ficus religiosa* leaves on experimentally induced wounds in rats.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Healthy Sprague dwaly rats of either sex weighing between 250-275 gm were used for the experiment. The institutional animal ethics committee approved the experimental protocol and animals were maintained under standard conditions in an animal house approved by the committee for the purpose of control and supervision of animals (CPCSEA).

EXTRACTION OF LEAVES

The leaves of *Ficus religiosa* (FR) were collected from the rural areas of Bangalore, Karnataka, India in the month of October 2007 and were authenticated by Mrs. Manjula Srinivasan, Head of Botany Department, Krupanidhi Institutions, Bangalore. The leaves were shade dried, powdered and stored in airtight container until further use. Around one kg of the leaves was extracted using a hydro alcoholic mixture (75% methanol) as a solvent in a Soxhlet apparatus until complete extraction. The extracts were subjected to preliminary phytochemical screening (₅).

PREPARATION OF FORMULATION

The extract was formulated as 5% (w/w) and 10 % (w/w) emulsifying ointments (₆). FR 5% (w/w) in emulsifying base was used as a low dose and FR 10% (w/w) was used as a high dose for topical application.

EXCISION WOUND MODEL (,)

The animals were anesthetized using ether. An impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear of the anesthetized rat. The skin of that area was shaved and was excised to full thickness to obtain a wound area of about 500 mm². The animals were divided into four groups and they were treated as follows: Group I: emulsifying base (control), Group II: standard nitrofurazone 0.2% (w/w) ointment, Group III: 5% FR ointment (low dose) and Group IV: 10% FR ointment (high dose).

The ointments were applied once daily until complete healing of wound. The wound area was measured on a millimeter scale graph paper. The percentage of wound healing was calculated on pre determined days post wounding. Falling of scar was taken as the endpoint for complete epithelization and the days taken for this was considered as period of epithelization. The wound contraction and period of epithelization were measured in all groups.

INCISION WOUND MODEL (,,)

In the incision wound model two para vertebral straight incisions of 6 cm were made on either sides of the vertebral column, homeostasis was achieved by blotting the wound with a cotton swab dipped in saline and the wound was closed by means of interrupted sutures at equidistance 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0 day to 9th post-wounding day. The wound breaking strength was estimated

on the 10th day by continuous, constant water flow technique. The tensile strength in each group is determined.

BURN WOUND MODEL ()

The animals were anesthetized and grouped as in the incision model. Partially thickened burn wounds were inflicted by pouring hot molten wax at 800 C on the shaven back of the rat through a cylinder of 300 mm² circular opening. The wax was allowed to remain on the skin till it solidified; the cylinder was then removed with the wax adhering to it, which left a partial thickness circular burn wound. Immediately after injury and on subsequent days, the ointments were applied as mentioned above in the incision wound model.

STATISTICAL ANALYSIS

Results are expressed as mean \pm SEM. The comparisons between experimental groups were made using one way analysis of variance (ANOVA) followed by Dunnet test. $P < 0.05$ was considered significant.

RESULTS

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

The phytochemical analysis of the hydro alcoholic extract of FR revealed the presence of tannins, sterols, saponins, flavonoids, carbohydrates and proteins.

EFFECT ON EXCISION AND INCISION WOUND

Both high as well as low concentration of *Ficus religiosa* (FR) produced a significant decrease in period of epithelization when compared to control ($P < 0.01$). Treatment with Standard Nitrofurazone 0.02% also produced significant reduction in the period of epithelization ($P < 0.01$). All the treatments also showed significant decrease in wound contraction (50%) as compared to control ($P < 0.001$). It was also found that the high dose (10 %) of FR was comparatively more effective than low dose (5%) of FR in reducing the epithelization period (Table 1).

The breaking strength of 10 days old incision wound was increased by all treatments. The high dose of FR was more effective than low dose and standard in increasing the breaking strength of the incision wound (Table 1).

Figure 1

Table 1: Effect of (FR) on the period of epithelization and wound contraction 50% in excision wound model and breaking strength in incision wound model (All values are mean \pm SEM, n=6, **P

Groups	Excision wound		Incision wound
	Epithelization period (days)	Wound Contraction-50% (days)	Breaking strength
Control (Emulsifying base)	21.00 \pm 0.25	10.83 \pm 0.40	411.66 \pm 14.47
Standard Nitrofurazone 0.02%	18.83 \pm 0.25**	8.50 \pm 0.34**	463.33 \pm 11.94*
FR (5%) (Emulsifying base)	17.33 \pm 0.21**	7.83 \pm 0.30**	518.33 \pm 11.37**
FR (10%) (Emulsifying base)	14.83 \pm 0.40**	6.50 \pm 0.42**	641.66 \pm 11.94**

EFFECT ON BURN WOUND

Like the excision wound model, application of FR (5%), FR (10%) as well as Nitrofurazone 0.02% topically shortened the period of epithelization significantly (P < 0.001) and also produced a significant decrease (P < 0.001) in wound contraction-50% (days) when compared to control. The high dose of FR was found to be more effective when compared to low dose of FR and standard. (Table 2).

Figure 2

Table 2: Effect of (FR) on the period of epithelization and wound contraction 50% in burn wound model (All values are mean \pm SEM, n=6, **P

Groups	Excision wound	
	Epithelization period (days)	Wound Contraction-50% (days)
Control (Emulsifying base)	21.16 \pm 0.30	11.33 \pm 0.33
Standard Nitrofurazone 0.02%	19.00 \pm 0.36**	9.16 \pm 0.16**
FR (5%) (Emulsifying base)	18.10 \pm 0.16**	8.33 \pm 0.33**
FR (10%) (Emulsifying base)	15.16 \pm 0.60**	6.16 \pm 0.31**

DISCUSSION

The present study was undertaken to evaluate whether *Ficus religiosa* L leaves promote wound healing in experimentally induced wounds in rats. The results of the present study substantiate the use of *Ficus religiosa* L leaves in folklore medicine for the treatment of wounds. The emulsifying base

containing hydroalcoholic extract applied topically promoted the breaking strength, wound contraction and period of epithelization in different models of experimental wounds.

Collagenation, wound contraction and epithelization are crucial phases of wound healing. The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually either promote or depress one, other or all phases of healing. Growth hormone is known to promote the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue, cartilage. The collagen synthesis is stimulated by various growth factors (₁₃). Growth hormone is also known to promote the proliferation of fibroblasts (₁₄) and fibroblast proliferation form the granulation tissue. The exact mechanism(s) by which *Ficus religiosa* L leaves increased the granuloma tissue weight and breaking strength of granulation tissue cannot be explained with the present data. Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting the DNA synthesis (₁₅). Several antioxidants such as vitamin C, metronidazole and vitamin E are reported to increase the wound healing (₁₆). The wound healing activity of *Ficus religiosa* leaves extract can be attributed to the presence of their phyto constituents which may be active individually or it could be a synergistic activity of these constituents. It is speculated that tannins by virtue of its anti oxidant, anti inflammatory, astringent, and antimicrobial properties could be one of the contributors for the wound healing effect of the plant (₁₇). Moreover, antioxidant nature of sterols and the flavonoids of *Ficus religiosa* could also be responsible for wound healing activity (₁₈). From the results of this study it can be concluded that the hydro alcoholic extract of *Ficus religiosa* has significant wound healing activity in all the three models i.e. excision, incision and burn wound models.

To conclude, leaves of *Ficus religiosa* possess good wound healing activity when applied locally. The high dose of the extract was found to be more effective topically than the low dose. Further, isolation of active constituents from the extracts of the leaves may bring about the development of a new wound-healing agent.

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