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Exploration of Healing Promoting Potentials of Roots of *Ficus religiosa*

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Abstract: Backgrownd: *Ficus religiosa* (Family-Moraceae) which is commonly known as Pepal tree is abundantly distributed throughout in India. *Ficus religiosa* leaves are reported to have wound healing, Anti-inflammatory, analgesic, anti lipid- peroxidation activity. Hence the present study was aimed to investigate the wound healing activity by excision and incision wound models. **Materials and Methods:** The wound-healing activity of *Ficus religiosa* extracts in ointment form (5 and 10%) was evaluated on Wistar albino strain rats of either sex. Povidine iodine 5% was used as Standard drug. The healing of the wound was assessed by the rate of wound contraction, period of epithelialization and skin breaking strength. **Results:** Both the extracts as ointments (5 and 10%) of *Ficus religiosa* root extracts promoted the wound-healing activity significantly in all the wound models studied. High rate of wound contraction, decrease in the period for epithelialisation, high skin breaking strength et al high hydroxyproline content were observed in animals treated with 10% root extract ointment when compared to the control group of animals. **Conclusion:** Therefore, root extracts of *Ficus religiosa* in the form of 10% ointment promotes wound-healing activity better than the former concentration, 5%.

Key words: Wound healing activity, *Ficus religiosa*, ethanolic extracts

INTRODUCTION

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular anatomic or functional continuity of living tissue (Ramzi *et al*., 1994). Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favorably alter the healing process (Enoch and Price, 2004). Although many indigenous tribes around the world have long suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds (Kurian, 1995).

Some of plants possessing prohealing activity have been scientifically analyzed. The wound healing potential of *Tridax procumbens* (Udupa *et al*., 1995), *Trigonella foenumgraecum* (Taranalli and Kuppast, 1996), *Leucas lavandulaefolia* (Saha *et al*., 1997) and *Aloe vera* (Chithra *et al*., 1998) have shown promising healing activity.

Research on wound healing drugs is a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda, the Indian traditional system of medicine. Most of these drugs are derived from plant origin. Some of these plants have been screened scientifically for the evaluation of their wound healing activity in different pharmacological models and patients but the potential of most remains unexplored. With a view to increasing the wide spectrum of medicinals usages, the present day reqiures a new biologically active ointment which exhibit wound healing activity as local applications.

Ficus religiosa (Family-Moraceae) which is commonly known as Pepal tree is abundantly distributed through out in India. Even though the bark having wound healing activity, (Choudhary, 2006), anti-inflammatory, analgesic, anti lipid- peroxidation activity (Sreelekshmi *et al*., 2007) and have purgative properties (tender shoots) (Warrier *et al*., 1995) but as per our knowledge there is no such literature till available for wound healing activity of root extracts of title plant in the form of ointment. Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (Savanth *et al*., 1998). Therefore, the present study was aimed to do the wound healing activity of root extract of Ficus Religiosa in the form of ointments.

MATERIALS AND METHODS

Materials: The root of *Ficus religiosa was* collected from adjoining areas of Modasa (Sabarkantha), in July, 2009 and was authenticated by Associate Professor

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Dr. M. S. Jangid, Department of Botany, College campus, Modasa, Hemchandracharya North Gujarat University, Patan (Gujarat), India, by carrying out macroscopic and microscopic evaluation.

Preparation of the root extract: Root of the plant was dried in shade. The dried root was powdered (3 kg), defatted with petroleum ether (60-80°C) and soaked in ethanol (95%) and kept aside for 4 days. After 4 days, the ethanolic layer was decanted off. The process was repeated for four times. The solvent from the total extract was distilled off.

The preliminary phytochemical analysis: The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic extract (Trease and Evans, 1987).

Animals: Wistar albino rats of either sex weighing between 180 and 200 g were obtained from Jay Research Foundation, Vapi. The study was approved by the Institutional Ethics Committee for animal experimentation VBTCP, Umrakh and all the procedures on animals were carried out as per CPCSEA guidelines, India. These animals were used for the wound healing activity studies. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60±5% relative humidity and 12 h light dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study.

Excision wound model: Under light ether anaesthesia an impression of 500 sq mm was made on the shaved back of the rat as described in Fig. 1 (Morton and Malone, 1997). The skin of the impressed area was excised carefully. Animals are kept in separate cages. The day on which wound was made consider as ay '0' (Zero).

Animals divided into four groups of each with 5 animals. Group A considered as control and treated with simple ointment (eg., Bees wax, Cetosteryl alcohol etc.), group B considered as standard and treated with 5% w/w Povidine iodine ointment, group C and group D were *Ficus religiosa* treated group and applied ointment 5% and 10% respectively (Table 1). The percentage of wound closure was recorded on day 4, 8, 12 and 16. Wound area was traced and measured planimetrically with the help of sq mm graph paper. Number of days required for falling of the eschar without any residual raw wound gave the period of epithelization (Fig. 1).

Incision wound model: The rats were anesthetized by administering ketamine (0.5 mL kg^{-1} b. w. i.p.). Incision wounds of about 6 cm in length and 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm intervals (Fig. 2). Surgical thread (No. 000) and a curved needle (No. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with topical application of the Ointments as described above, for the period of 10 days. The wounding day was considered as

Fig. 1: A circular excision wound on the day zero. Wound after 8 day treatment

Table 1: Effect of ethanolic root extracts ointment of *Ficus religiosa* on Excision wound parameters

	% Wound contraction				
Groups	4th Day	8th Day	12th Day	16th Day	Epithelialization time (days)
Control (A)	20.84±2.28	40.89±1.33	57.88±1.47	83.51±0.91	23.5±0.63
Standard povidine iodine 5% (w/w) (B)	31.09±.06***	54.48±1.20***	79.79±0.98***	98.03±0.53***	18±0.54***
Ficus religisa 5% (w/w) (C)	23.38±2.14*	46.57±3.56**	59.46±4.49***	88.69±0.74***	20.2±0.58*
Ficus religiosa 10% (w/w) (D)	31.78±3.86***	61.13±0.46***	86.45±3.00***	97.34±0.64***	19±0.60***

The values are expressed as Mean±SEM, n = 5 in each group. If *p<0.05, **p<0.01 and ***p<0.001 vs. control

Fig. 2: Incision wound on the day zero

Fig. 3: A completely healed incision

day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day (Fig. 3) and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer (Fig. 4) on the 10th day reported in Table 2 (Singh *et al*., 2006).

Tensile strength was calculated using the following formula (Reddy *et al*., 2008):

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

Estimation of biochemical marker: Circular wound with approximate area of 500 mm^2 was created using the procedure described in excision wound model. The

Fig. 4: Tensiometer for the measurement of tensile strength of skin

wounds were treated with topical application of ointments as described above for 10 days. The scab was removed on 11th day and dried in oven at 110°C. The hydroxyproline content in dried scab was determined by extracting hydroxyproline from scab using concentrated Hydrochloric acid followed by reaction between amino groups of hydroxyproline with p-dimetylaminobenzaldeyde to develop red colour. The red colour thus measured on Spectrophotometer at 558 nm using the method described by Bergman and Loxley (1963).

Statistical analysis: Results obtained from the three wound models have been expressed as Mean±SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test (Mukherjee *et al*, 2000).

RESULTS

Phytochemical analysis: Qualitative phytochemical analysis revealed presence of tannins especially hydrolysable tannins and alkaloids and saponins. The presence of alkaloid was confirmed by performing TLC and spraying with Dragendorff's reagent.

Excision wound model: Topical application of *Ficus religiosa* increased the percentage of wound contraction and completed wound healing by 16th day, which indicates rapid epithelization and collagenization. In fact, topical administration of *Ficus religiosa* extract accelerated the progression of wound healing by 12th day, i.e. (86.45±3.00***) $p<0.001$ compared with control

Table 2: Effect of ethanolic root extracts ointment of *Ficus religiosa* on breaking strength (g) in incision wounds

Groups	Breaking strength(g)
Control (A)	270.7±4.38
Standard Povidine iodine 5% w/w (B)	586.0±8.84***
Ficus religiosa (5%) extract (C)	493.9±7.25**
Ficus religiosa (10%) extract (D)	572.2±6.93***

The values are expressed as Mean±SEM, n = 5 in each group. If *p<0.05, **p<0.01 and ***p<0.001 vs. control

Table 3: Effect of ethanolic root extracts ointment of *Ficus religiosa* on Hydroxyproline content in the scab of excision wound

Groups	Hydroxyproline (µg/500 mg)
Control (A)	9.20±0.56
Standard povidine iodine 5% w/w (B)	32.37±0.63***
Ficus religiosa (5%) extract (C)	19.45±0.33**
Ficus religiosa (10%) extract (D)	29.99±0.36***

The values are expressed as Mean±SEM, n = 5 in each group. If *p<0.05, **p<0.01 and ***p<0.001 vs. control

(57.88±1.47) with 10% extract. It also reduced the epithelization time from 23.5±0.63 to 19±0.60 days, p<0.001 compared with control. Hydroxyproline levels in extract treated groups were significantly increased. Povidine iodine also showed significant effect (79.79±0.98) on 12th days i.e., p<0.001 as compared with control (Table 1).

Incision wound model: The breaking strength of the incision wounds was increased in drug treated groups to significant extent, i.e., 270.7±4.38 in control was increased up to 493.9±7.25 with 5% Extract and up to 572.2±6.93 with 10% extract. The results are also comparable to standard drug Povidine iodine (Table 2).

Biochemical marker estimation: The results indicated that the animals treated with ointment containing 5% (w/w) and 10% (w/w) total ethanolic extract have good wound healing activity, hence, the biochemical marker such as hydroxyproline content in the scab of excision wound created in the animals treated with stated extracts was determined on the 11th day. The animals treated with ointment containing 10% (w/w) ethanolic extract indicated significantly high (p<0.001) levels of hydroxyproline (29.99±0.36 µg/500 mg) as compared to control (9.20±0.56 µg/500 mg) (Table 3).

DISCUSSION

Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue (Phillips *et al*., 1991). From the above result of excision wound model it is evident that on the day 4th to 8th day, there was no significant increase in wound contraction in both the groups compared to control groups. However, the wound contraction was increased significantly (p<0.001) on the day 12th onwards.

Hydroxyproline is one of the biomarkers indicating wound healing process, as the content of the same is increased on 10th day. The increased hydroxyproline content in the scab of the animals treated with 5% (w/w) and 10% ethanolic extracts supported the wound healing process. However, 10% extract was better than 5% extract. The tensile strength with incision model showed maximum activity for wound healing and the result was singnificant (p<0.001), i.e., 572.2±6.93 with 10% extract in comparision to control 270.7±4.38. The standard drug povidine iodine was also comparable and significant (p<0.001). Several phytoconstituents like alkaloids (Ansel, 2008) and saponins (Mukherjee, 2002) are known to promote wound healing process due to their antioxidant and antimicrobial activities. The study reveals that both 5 and 10% extracts treated groups possesses good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like, alkaloids, saponins and tannins present in it.

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