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Evaluation of Wound Healing Activity of *Ficus bengalensis* Shoot Tips Extract

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The objective of present study is to establish the wound healing activity of Ethanolic shoot tip extract of *Ficus bengalensis* (Family: *Moraceae).* Excision model was performed to evaluate the wound healing activity in Rabbits .In excision model percentage wound contraction and period of epithelialization was determined for the extract. Povidone iodine ointment was used as Reference standard drug. From the observation of excision model, Ethanolic extract of *Ficus bengalensis* (400mg/kg) was found to have greater wound healing activity in terms of percentage wound contraction and period of epithelialization than that of other groups. In conclusion, our findings suggest that Ethanolic extract of *Ficus bengalensis* possesses better wound healing ability and may be due to the presence of phytochemicals like glycosides, tannins, flavanoids, saponins present in plant extract.

Key words: *Ficus bengalensis*, Excision wound , Povidone iodine .

The complex biology of wound healing is an area in which theoretical modeling has already made a significant impact .Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. The wound repair process has three orderly but temporally overlaid stages i.e. inflammation, cell proliferation and tissue regeneration[1]. Wound healing is a process which is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phases followed by synthesis of collagen and other extracellular macromolecules which are later remolded to form scars[2]. Therefore, tissue repair and wound healing are the complex processes that involve a series of biochemical and cellular reactions[3-5]. There are several reports stating that the extracts of several plants have wound healing properties[3,6,7].The need for safer and effective wound-healing agents and the lack of enough scientific data to support the claims prompted the present study.

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. *Ficus*

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bengalensis Linn is a large evergreen tree found throughout forest tracts of India. In traditional system of medicine various plant parts such as stem bark, root bark aerial roots, vegetative buds, leaves, fruits and latex are used in dysentery, diarrhea, diabetes leucorrhoea, menorrhagia, nervous disorders, astringent, anti-diabetic, anti-stress, anti-dairrhoeal and immunomodulatory properties [8]. At present Wound healing activity by Excision model was performed in rabbits *in vivo* conditions[9].

MATERIAL AND METHODS

Collection

Many parts of the tree F*icus.bengalensis* were found to poccess various biological activities yet shoot tips of F*icus.bengalensis* was not reported for its activities, hence shoot tips are taken to evaluate for its therapeutic effect. Shoot tips of F*icus.bengalensis* were collected from the surrounding villages of Kakinada and the plant was authenticated by Dr.Raghuram, taxonomist. The shoot tips was dried in shade, and then pulverized into powder.

Preparation of Ethanolic Extract of *Ficus bengalensis* shoot tips

The freshly collected shoot tips of the plant were cleared from dirt then, dried under shade for about 15days and then coarsely powdered in a mechanical grinder. The powder was macerated with ethanol for 5 days, filtrate was collected & concentrated. The concentrated product was dried using desiccators with anhydrous calcium chloride. The percentage yield of the extract was 4.8% w/w.

In-vivo Study

Experimental Animals

Rabbits of either sex weighing 1.6-2.2kg were used for the study. Before and after surgery the animals were housed individually in polypropylene cages. They were allowed to feed on a standard, commercial pellet diet supplemented with fresh vegetables and water *ad libitum*. The animals were maintained in a holding room illuminated with 12 hrs light/dark cycles. Room temperature was set at 23±2°C with humidity of 45% to 55%.

Excision wound model : Hairs of lower back and left flank of the test animals were fully shaved and cleared, the desired area was locally sterilized and anaesthetized with the subcutaneous injection of lidocaine (2%)[10]. A full thickness of the excision wound of circular area 500 mm ^{2} and 0.2 cm depth was created[11]. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a size No.15 scalpel blade and a forceps. Animals were divided into 4 groups of 3 each.

Group I Rabbits were treated topically with povidone iodine ointment (betadine) as a standard healing agent[12].

Group II Rabbits were treated as control group were treated with simple ointment base[13]

Group III Rabbits were treated with ethanolic extracts of *Ficus.bengalensis* of two doses (200mg/ kg b.w/day)[14].

Group IV Rabbit were treated with ethanolic extracts of *Ficus.bengalensis* of two doses (400mg/ kg b.w /day.) respectively[14].

Application of drugs was done using a sterile swab, twice daily. Test animals with infected wound were excluded from the study. All ethical issues were considered in surgery procedure and during the treatments. The area of the wounds on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound on the first day. Healing percentages on different days of treatments were calculated as the difference between the initial wound surface area and that on the day of measurement. They were observed thoroughly for epithelization and contraction of wound. Number of days required for falling of scab without any residual raw wound gave the period of epithelization [15].

The percentage protection was calculated on the 21st day by using the following formula and tabulated[16]

Percentage protection = 100- [Final diameter(cm)X 100/ initial diameter(cm)]

Statistical analysis

The mean value of SEM was calculated for each parameter. Results were statistically analysed by one-way-analysis of variance (ANOVA) followed by post hoc dunnet's test $p<0.001$ was considered statistically significant.

RESULTS

The wound healing contracting ability of Ethanolic shoot tips extract of *Ficus bengalensis* in different concentrations on excision wound model was significantly greater than that of the control group. The 400mg/kg extract ointment treated groups showed significant wound healing from 3rd day onwards which was comparable to that of standard drug i.e. Betadine treated group. The wound closure time was lesser, as well as the percentage of wound contraction was greater with 400mg/kg dose of *Ficus.bengalensis* extract and 100% contraction was observed in (17.7±0.14) days,

Table 1. Percentage wound contraction and period of epithelialization in excision wound model

Group	Percentage (%) of wound healing on the day							Period of Epitheliali zation
	3rd	6th	9th	12th	15h	18th	21st	
Control	2.24±5.77	8.1±0.37	13.2±0.23	19.2±0.1	25±0.15	36.3±0.5	48.3±0.4	24.1±0.4
Ficus bengalensis (200mg/kg)	2.65±0.01	13.6±0.0*	31±0.4*	45.2±.2*	64.9±.2*	73.7±0.3*	87.2±0.3*	18.9±0.38*
Ficus. bengalensis (400mg/kg)	3.07±0.07	18.7±0.3*	35.4±0.3*	49.8±.3*	72.4±.6*	84.6±0.3*	97.3±.66*	17.7±0.14*
Betadine (povidone iodine)	3.32±0.03	23.4±0.3*	38.7±0.6*	52.7±0.*	74.1±.8*	86.5±1.4*	98.5±0.1*	14.8±0.6*

Values are expressed as Mean ±SEM; n=3 animals in each group; * *P* d"0.001 when compared to control

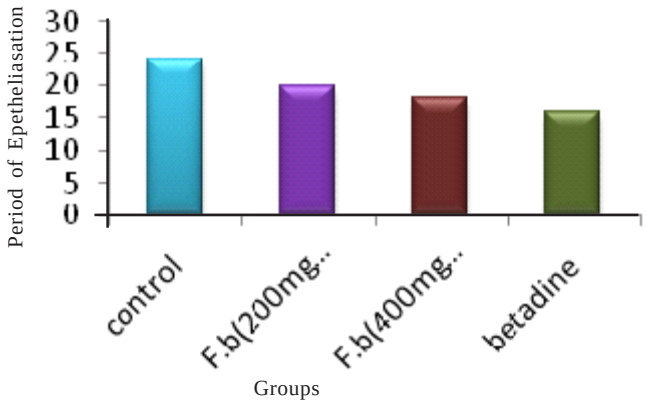


Fig. 1. Measurement of period of Epetheliasation in excision wound model

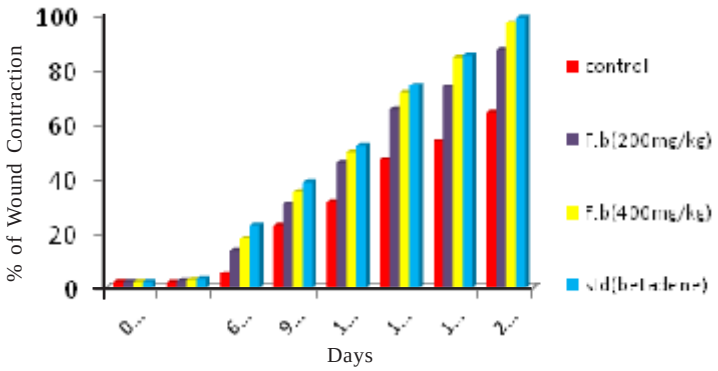
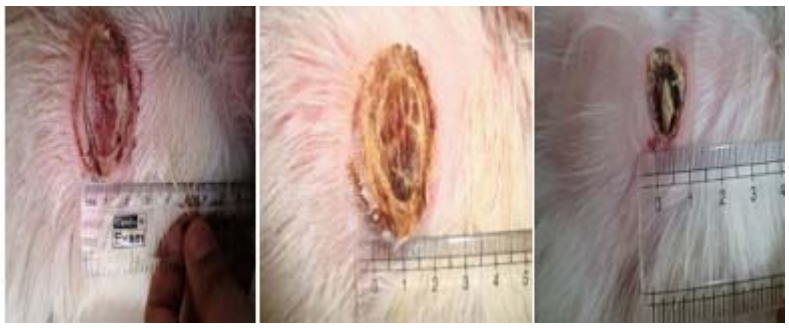


Fig. 2. Measurement of percentage wound contraction in Excision model

Plate 1. Rabbit with wound on 0-day

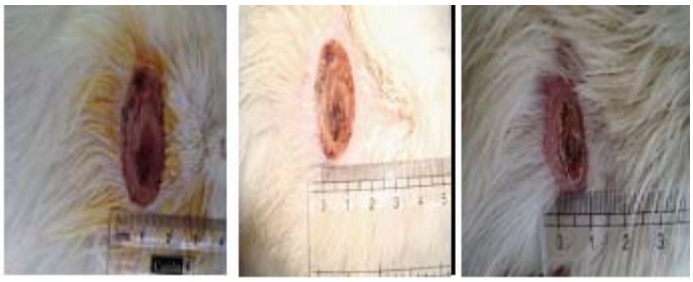
3rd day 9th day 15th day

Plate 2. Effect of *Ficus bengalensis* extract (200mg/kg) on wound



3rd day 9th day 15th day

Plate 3. Effect of *Ficus bengalensis* extract(400mg/kg) on wound



3rd day 9th day 15th day

Plate 4. Effect of Betadine on wound

which was almost similar to that of betadine treated group (14.8±0.6) days. The 200mg extract group of animals showed significant wound contraction from 6th day onwards and achieved 100% wound closure in 18.9±.38 days as shown in Table 1, FIG 1, 2. The results of present study revealed that both concentrations (200mg/kg, 400mg/kg) of Ethanolic extract of *Ficus bengalensis* shoot tips have significant wound healing activity in excision model. Image 1-4 indicates wound appearance before and after treatment with extract of different doses and standard drug.

DISCUSSION

In the present study *Ficus. bengalensis* found to be the most effective against wound healing [17]. Two or more of the common phytoconstituents like alkaloids, tannins, phenols, glycosides and flavonoids were detected in these active extract [18]. In excision wound model the shoot tips extract of *Ficus bengalensis* showed significant increase in percentage wound closure by enhanced epithelialization. This enhanced epithelialization may be due to the effect of *Ficus bengalensis* extract on enhanced collagen synthesis. From the present study, it can be concluded that Ethanolic extract of *Ficus bengalensis* shoot tips in both concentrations show significant wound healing activity.

CONCLUSION

The present study demonstrated that the Ethanolic extract of *Ficus. bengalensis* have properties of promoting wound-healing activity compared with placebo control. The study provides scientific evidence for further evaluation of *Ficus. bengalensis* in the topical treatment and management of wounds.

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