

Wound–healing activity of ethanolic and aqueous extracts of *Ficus sarmentosa* bark

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ABSTRACT

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. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. Traditionally, *Ficus sarmentosa* is used for wound healing. Since no detailed scientific data are available regarding the wound-healing activity of *F. sarmentosa*, the present study was designed to explore the same. The wound-healing efficacy of ethanolic and aqueous extracts of *F. sarmentosa* was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization, and tensile strength of incision wound. Student's *t* test was used to analyze the results obtained from the present study and $P < 0.05$ was considered significant. Both the ethanolic and aqueous extracts of *F. sarmentosa* were found to possess significant wound-healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction and skin-breaking strength. The present study has demonstrated that the ethanolic and aqueous extracts of *F. sarmentosa* have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control.

Keywords: Excision wound model, *F. sarmentosa*, incision wound model, tensile strength

INTRODUCTION

Wounds are physical injuries that result in an opening or breaking of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical stability and disturbed functional status of the skin. Repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation, and migration of different cell types [1] 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. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and

undergo contraction using a mechanism similar to that in smooth muscle cells. [2] Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction, a number of herbal products are being investigated at present. Various herbal products have been used in management and treatment of wounds over the years. [3]

Ficus sarmentosa (Family: Moraceae) is a fig tree with edible fruit. *F. sarmentosa* is native to China, Eastern Asia, Indian Subcontinent and Indo-China region. Traditionally, it is used for wounds, fever, swollen joints, inflammations, and ulcers. Edible bark powder is taken to cure boils and secrete more milk during delivery. Root extract is used in malaria. All the parts of the plant have astringent, anti-inflammatory, antiarthritic, and antidiarrheal activities. A bath made from the fruit and bark is a cure for leprosy. Latex is drunk to cure fever. Raw fruits are eaten in diarrhea. Young fruit juice is applied in forehead to relieve headache. Young twigs are fed to cattle for facilitating the discharge of placenta. [4] Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe

complications. The need for safer and effective wound-healing agents and the lack of enough scientific data to support the claims made in ancient literature prompted the present study.

Material and methods

The plant of *F. sarmentosa* was collected in the month of August, 2016 from the local area of Chamoli district and identified and authenticated by Botanical Survey of India, Northern Regional centre, Dehradun (BSI) with the Accession number 1127802, 1127803. A voucher specimen has been preserved in the department for further verification.

Extraction

The bark of *F. sarmentosa* was dried under shade, crushed into small pieces, and powdered. The powder was loaded into Soxhlet extractor and was subjected to successive extraction with petroleum ether, benzene, chloroform, ethanol, and water to get different extracts. The ethanolic and aqueous extracts were concentrated to dryness using Rotary evaporator, giving yield as 4.10% w/v and 4.42%, respectively, and preserved in a refrigerator. Aliquot portions of the ethanolic and aqueous extracts of *F. sarmentosa* were weighed and suspended in an

appropriate volume of Tween 80 (2% v/v) for use on each day.

Acute Toxicity Study of the Extract

Female Albino Wistar rats weighing 200 to 220 g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines.[5] The animals were fasted overnight with water *ad libitum*. The starting dose of 5 mg/kg of both ethanolic and aqueous extracts was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2 000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Table - 1 Wound-healing effect of *Ficus sarmentosa* in excision wound model

Parameter	Wound area (mm ²) and percentage of wound concentration		
Post wounding days	Control	AEFS (200mg/kg)	EEFS (200mg/kg)
Day 0	503.66±2.9857	503±3.2151	505.66±2.1554
Day 2	478.33 ±3.7749 (5.02%)	469.66±1.202 (6.62%)	465.331±1.3335* (7.97%)
Day 4	425.33 ±1.3336 (15.55%)	393.33±0.989** (21.80%)	366±2.7814 (27.61%)
Day 8	338±1.1549 (32.89%)	292.66±1.3335** (41.81%)	268.66±1.7641** (46.86%)
Day 12	211.66±1.202 (57.97%)	165±1.5278** (67.19%)	125±1.3418** (75.27%)
Day 16	77.33±1.7641 (84.64%)	22±1.1549** (95.62%)	13.66±1.202** (97.29%)
Day 18	24.33±1.202 (95.16%)	5.66±0.9546** (98.87)	00±00** (100%)
Day 20	9±0.8565 (98.21%)	00±00** (100%)	00±00** (100%)
Period of epithelialization (day)	21.5±0.3416	18.33±0.2108**	17.16±0.1666

n=6, value are expressed as a mean± SEM; *P<0.01;**P0.001Significant as compared with control;AEFS: Aqueous extract of *Ficus sarmentosa*; EEFS: Ethanolic extract of *Ficus sarmentosa*

Preliminary Phytochemical Studies

The different extracts were then subjected to qualitative phytochemical screening for the

identification of the phytoconstituents. Although petroleum ether, benzene, and chloroform does not show any appreciable tests for the presence of

different phytoconstituents, ethanolic and aqueous extracts showed positive tests for the presence of glycosides and flavonoids. In order to follow the reasonable method of isolation of active ingredients from the plants, "activity guided" fractionation was followed. To achieve this, wound-healing activity of ethanolic and aqueous extracts was carried out at a dose level of 200 mg/kg.

Experimental Animals

The Institutional Animal Ethics Committee approved the use of animals for the present study (Ethical clearance number: 1145/a/07/CPCSEA/2011/6). Healthy Wistar albino rats of both sexes weighing 200 to 220 g was used for the study. They were individually housed and were allowed free access to standard pellet diet and water *ad libitum*. Animals were periodically weighed before and after the experiment. The rats were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine anesthesia (120 mg/kg). Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and were replaced.

Wound-healing Activity

Excision and incision wound models were used to evaluate the wound-healing activity of aqueous and ethanolic extracts of *F. sarmentosa*.

Excision Wound Model

Animals were anesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malone [6]. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500 mm² and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The entire wound was left open [7]. The animals were divided into two groups of six each. Group 1 animals were topically treated with the simple ointment base I.P. as a placebo control. The animals of group 2 were topically treated with the 10% ointment of the aqueous extract of *F. sarmentosa* formulated in simple ointment base I.P. till complete epithelialization [8]. The wound closure rate was assessed by tracing the wound on days 0, 2, 4, 8, 12, 16, 18, and 20 post-wounding using transparency sheets and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar

without any residual raw wound gave the period of epithelialization.

Incision Wound Model

As with the above model, rats were anesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, 6 cm in length, was made through the skin and cutaneous muscle on the back, as described by Ehrlich and Hunt [9]. After the incision, surgical sutures were applied to the parted skin at intervals of 1 cm. The wounds were left undressed. The rats were given *F. sarmentosa* extract (dissolved in tween-80, 0.5%) orally at a dose of 200 mg kg⁻¹ day⁻¹. The controls were given with tween-80 0.5% only. The sutures were removed on the eight post-wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method described by Lee [10]. The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light-weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Statistical Analysis [11]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's *t* test and $P < 0.05$ were considered significant.

Result

Acute Toxicity Studies

In the acute toxicity studies, no signs of toxicity or mortality were observed at 2000 mg/kg dose level. So, 200 mg/kg b.w. dose was taken as the therapeutic dose.

Excision Wound Model

The significant increase in the wound-healing activity was observed in the animals treated with the *F. sarmentosa* extracts compared with those who received the placebo control treatments. In the excision wound model, *F. sarmentosa*-treated animals showed a significant reduction in the wound area ($P < 0.001$) [Figure 1] and epithelialization period

[Table 1]. The ethanolic extract showed healing in 17.16 days, whereas the aqueous extract showed healing in 18.33 days as compared with 21.50 days of control.

Table 2 Wound-healing effect of *Ficus sarmentosa* in Incision Wound Model

Parameter	control	AEFS (200 mg/kg)	EEFS (200mg/kg)
Skin –breaking strength (g)	283.5± 4.0484	447.66 ± 2.70448*	466.66 ± 2.459*

Table shows the effects of the ethanolic and aqueous extracts of *F. sarmentosa* administered orally at a dose of 200 mg kg⁻¹ day⁻¹ for 10 days on wound-healing activity in rats inflicted with incision wound. In the incision wound model, a significant increase in the wound-breaking strength was observed with the ethanolic and aqueous extracts of *F. sarmentosa* when compared with the control. n = 6, values are expressed as mean ± SEM; *P<0.001 significant as compared with control; AEFS: Aqueous extract of *Ficus sarmentosa*; EEFS: Ethanolic extract of *Ficus sarmentosa*.

Discussion

Wound healing is a highly complex, but orchestrated cascade of events that can roughly be divided into three overlapping phases—*inflammation, granulation tissue formation, and remodeling of the extracellular matrix.* These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phases of wound repair involve inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans, which are heterogeneous, nonfibrillar components of the extracellular matrix. These complex macromolecules are made up of a protein core linked covalently to linear heteropolysaccharides, the glycosaminoglycans (GAGS). Proteoglycans and GAGS have been shown to play important roles in all the above-mentioned events of wound healing. [12] Biological activities in skin are due to its interaction with various binding proteins. In the tissue repair process, inflammatory cells promote the migration and proliferation of endothelial cells leading to neovascularization of connective tissue cells which synthesize extracellular matrices including collagen resulting in re-epithelialization of wounded tissue. [13] The increased tensile strength may be due to increased collagen concentration and stabilization of fibres. The wound - healing property of *F. sarmentosa* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. The early tissue approximation and increased tensile strength of the incision wound observed in our study may have been contributed by the phytoconstituents of *F. sarmentosa*.

Conclusion

The present study has demonstrated that the ethanolic and aqueous extracts of *F. sarmentosa* have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control. Wound contraction and increased tensile strength support further evaluation of *F. sarmentosa* in the topical treatment and management of wounds.

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