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Healing promoting potentials of roots of *Ficus benghalensis* L. in albino rats

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ABSTRACT

Objective: To screen the wound healing activity of aqueous and ethanolic extract of roots of *Ficus benghalensis*. **Methods:** Both the extracts were evaluated for wound healing by three models *ie*. incision, excision and dead space wound. In incision and dead space the extracts were applied daily topically till the 10th post wounding days while in excision model it was till the complete epithelialization process. Standard group were administered Povidone iodine ointment topically daily. The breaking strength, percentage of wound contraction, period of epithelialization, dry granulation weight and hydroxyproline content were observed. **Results:** The result of the present study showed that both extracts were able to increase the breaking strength (incision model), decrease period of epithelialization, increase percentage wound contraction (excision model), increase hydroxyproline content (Dead space wound model) significantly compared with control group ($P<0.05$). **Conclusions:** Based on result we conclude that aqueous extract is more effective than ethanolic extract. However, it is needed more research to be carried out especially on toxicity studies of ethanolic extract.

1. 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 and anatomic or functional continuity of living tissue[1]. Wound healing studies are mainly aimed 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[2]. Wound healing process involves several steps such as coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength. During the formation of new tissue, endothelial cells proliferate and form new blood vessels. There are several reports stating that the extracts of several plants are used for wound healing properties[3-5].

Some of plants possessing prohealing activity have been scientifically analyzed. The wound healing potential of *Tridex procumbens*, *Trigonella foenumgraecum*, *Leucas lavandulaefolia* and *Aloe vera* have shown promising healing activity[6].

Ficus benghalensis Linn (Family-Moraceae) is commonly known as Banyan tree or vata or vada tree in Ayurveda. *Ficus benghalensis* is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks. This tree is considered to be sacred in many places in India[7]. Traditionally all parts of the plant are astringent, acrid, sweet, refrigerant, anodyne, vulnerary, depurative, anti-inflammatory, ophthalmic, styptic, antiarthritic, diaphoretic, antidiarrhoeal, antiemetic and tonic[8]. It is used in Ayurveda for the treatment of diarrhea, dysentery and piles, teeth disorders, rheumatism, skin disorders like sores and to boost immune system, as a hypoglycemic[9]. Bark contains tannins, wax, esters and glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirol-alpha-D-glucose and meso-inositol. Two flavonoid compounds, *viz*. 5, 7-dimethylether of leucopelargonidin 3-0-alpha-L-rhamnoside and 5,3-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were present in the bark of *Ficus benghalensis*[9,10]. Pharmacological evaluation has shown the various extract of *Ficus benghalensis* has shown anthalmintic[9], analgesic[10], anti-inflammatory[10],

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antioxidants[11], antidiabetic[12], Immunomodulatory[13] and antimicrobial[14] activity in experimental animals. The ethnomedicinal use of the roots of *Ficus benghalensis* in wound healing has not been systematically investigated so far. Therefore the present study was designed to determine the healing activity of roots in the wound model in rats.

2. Materials & methods

2.1. Materials

Roots of *Ficus benghalensis* L. growing in natural habitant in Modasa, Gujarat, India, was collected in October, 2010 and identified by Associate Professor Dr. MS Jangid, Department of Botany, Modasa, Hemchandra Gujarat University by carrying out macroscopic and microscopic evaluation and has been submitted in the institute for future reference purpose.

2.2. Preparation of the root extract

Dried and coarsely 500 g powdered roots of *Ficus benghalensis* was extracted with 90% (v/v) ethanol in soxhlet apparatus for 36 h and aqueous extract was prepared by using maceration technique of extraction. After filtration, the filtrate was concentrated on water bath using petridish. The temperature was maintained at 55 ℃. The powdered extract was dried and weighed.

2.3. The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic & aqueous extract[15-18].

2.4. High performance Thin Layer Chromatography (HPTLC) profile

Chromatography was performed on 3 cm ×10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F254 (Merck, Germany). Before using, the plates were washed with methanol and activated at 110 ℃ for 5 min. Samples were applied as 4 mm wide bands and 6 mm apart by using a Camag (Muttenz, switzerland) Linomat Ⅳ sample applicator equipped with 100 μL syringer. A constant application rate of 5 μL/s was used.

2.5. Animals

Wistar albino rats of either sex weighing between 180 and 200 g were obtained from Jai Foundation Research, Vapi (Gujarat). The study was approved by the Institutional Ethics Committee for animal experimentation Vidyabharti Trust College of Pharmacy, Umrakh, Gujarat (VBT/IAEC/10/12/30) 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. The ethanolic & aqueous extract of *Ficus benghalensis* was administered orally (*p.o.*) to all group of animals.

2.6. Incision wound model

The rats were anesthetized by administering ketamine (0.5 mL/kg bw. *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. 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 drug by oral administration as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day reported[19,20].

2.7. Excision wound model

A circular piece of full thickness [approximately 500 (mm. sup.2)] was removed from a predetermined dorsal area[21]. The wound were traced on 1-(mm.sup.2) graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in the wound area were calculated, giving an indication of the rate of wound contraction. The number of days required for falling of the eschar without any residual raw wound was determined as the period of epithelization.

2.8. Dead space wound model

The dead space wounds were created by making a small transverse incision in the lumber region on either side of vertebral column in each animal. Two polypropylene tubes (2.5 cm × 0.5 cm) were inserted subcutaneously one on either side of vertebral column and pushed cephal head for 3-4 cm for the final implantation to harvest the granulation tissue. The animals were treated with the extracts from 0 day to 9th post-wounding day considering wounding day as zero. Granulation tissue formed on the implanted tubes was carefully dissected out on the 10th post-wounding day and the tensile strength was measured by continuous constant water flow technique[20]. Mean value gives the breaking strength for a given group. The tissue was dried in oven at 60 ℃ for 24 hours and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the estimation of the hydroxyproline content in the tissue[22].

2.9. Hydroxyproline estimation

Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60-70 ℃