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Research Paper

Evaluation of Wound Healing on Ficus Retusa

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Wound is describe as disturbance of physiological continuity and structural of a living tissue. It may be created by physical, chemical, thermal, microbial, or immunological damage to the tissue. The Indian customary system of remedy, Ayurveda is support on observed knowledge of the observations and the understanding over millennia. In special classical texts, more than 1200 diseases are mentioned. More than 1000 medicinal plants, 64 minerals, metals, 52 animal and marine products to make well wounds. Plants have the massive possible for the supervision and management of wounds. In a good number of the countries, a large figure of plants are used by ancestral and tradition for the treatment of wounds and burns. These usual agents induce healing and regeneration of the lost tissue by an assortment of mechanisms. This *Ficus Retusa* extract is not only low-priced and affordable but are also secure. The occurrence of wide variety of life-sustaining constituents in plants has urged scientists to observe these plant lives with a view to conclude potential wound healing properties. Frequent pharmacological reports are available on number of plants employing dissimilar wound healing models and its underlying molecular method for the corroboration of their habitual claims and development of potent, safe and useful and worldwide acknowledged herbal drugs for wounds.

Key words: *Ficus Retusa*, Wound healing, Immunological, Remedy.

INTRODUCTION

Wound healing is the improvement of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells under the dermis begin to increase collagen making. Later, the epithelial tissue is regenerated[1]. Healing is the interface of a complex flow of cellular events that generate resurfacing, reconstitution and restoration of the tensile strength of injured tissue. Under the most supreme circumstances, healing is a systematic process, traditionally explained in term of three classic phases known as inflammation, proliferation and maturation [2]. The prevalence of wound complication has increased tremendously worldwide and has become a serious problem for public health[3]. A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively[4]. Wound healing involves various steps like coagulation, development of granulation tissue, coagulation and acquisition of wound strength. During the formation of new tissue endothelial cell proliferates and forms fresh blood vessel[5]. Herbal medicines are currently

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in require and their popularity is increasing day-by-day. Herbal plants are contains or produce large amount of chemical compounds that are needed by human body to care for illness or diseases or to promote health and well being[6]. As the people become aware of the potency and side effect of the synthetic drugs, there is an increase in interest in the natural remedies with a basic approach towards the nature. Today, estimated 80% of people in developing countries still rely on traditional medicines[7]. Plants are the principle source of raw material for plant based medicines since ancient times the traditional medicines are receiving great importance in the health care sector over the world.[8] This research is conducted by using a well known medicinal plant which is *Ficus retusa*. The plant occurs in India to southern China, in Taiwan, and through Malaya to Australia and New Caledonia. The plant occurs in India to southern China, in Taiwan, and through Malaya to Australia and New Caledonia[9].

MATERIAL AND METHOD

Animals

The study was carried out in rats of Wister strains of either sex weighing 150-200 gm. 2-3 months old. They were procured from animal house of the Jaipur College of Pharmacy (A Unit Of Modern Society For Education And Research, Jaipur); and were kept individually under standard laboratory condition. Food pellets and tap water were provided and libitum. Ethical clearance for experimental studies was obtained from institutional animal Ethical Committee, National institute of the institute under reg. No. 931/PO/ac/06/CPCSEA.

Plant material

Ficus Retusa ***(****Moraceae***) was purchased from local market and its indientity was confirmed. It was dried well to make powder. Coarse powder of the dried rind was prepared with the help of the grinder. Hydro alcoholic extract of the powereded drug was prepared by Soxhlet's apparatus.

Preparation of extract

The air-dried parts of the plants were powdered and extracted with 95% ethanol, chloroform per ether (40-60) and aqueous solvent systems by hot percolation method by using Soxhlet apparatus assembly at a controlled temperature. After complete extraction, marc was pressed to collect the micelle, mixed with the contents of RBF, filtered and concentrated to get the extract. The color and consistency of the extract was noted.

Extraction of plant

The air dried part of *Ficus retusa* were reducing to coarse powder. The dry powder of plants part (500 g) was subjected to successive solvent extraction

procedure using various solvents petroleum ether, chloroform, acetone and methanol in the increasing order of polarity. The solvents were evaporated under reduced pressure to obtain a semisolid mass and then vacuum dried to yield solid residues. The dried extracts were stored in air tight container until the time of use.

Testing for Animals:

Testing of CBC and Total Serum protein estimation and histopathological study (Skin biopsy) were done from Precision Path Lab, Jaipur.

- Skin Biopsy

Chemicals

Sulphuric acid, sodium hydroxide, fehling‘s solution (A and B), hydrochloric acid (HCl), Mayer‘s reagent, Dragendorff‘s reagent, ferric chloride, Ammonia solution, chloroform and dichloromethane were purchased & distilled water was prepared in the department of pharmacology, Maharishi Arvind Institute of Pharmacy Jaipur.

Determination of acute toxicity (LD50)

The acute toxicity of petrolium ether, mathanolic and aquiesous extract of plant *ficus retusa* were determined in wister rat. The animal were fasted overnight prior to the experiment, fixed dose method of OECD guidline no. 420; (Annexure-2d) of CPCSEA was adopted for this purpose.

Toxicity studies of petroleum ether and ethanolic extracts of *ficus retusa* were carried out on rats, when topically applied in a concentration of up to 5% did not show any toxic side effects or erythma on skin surface. Thus the prepared extracts were considered safe for topical administration.

Collection and Authentication of Plant:

Identification of the root of *ficus retusa* University of Rajasthan (Jaipur, Rajasthan).

Reference no. : **RUBL211420**

Plant is authenticated by Vinod kumar Sharma, Botanist, UOR, Jaipur. Material was shade dried at room temperature and powdered mechanically and passed through a sieve #40.

Experimental models

Excision Wound Model

Animals were under light ether anesthesia throughout the surgical procedure. And impression of 2.5 cm diameter (500sqmm) as described by morton was made after leaving at least 5 mm space from the ears. The skin of the impressed area was excised carefully to the complete thickness and a wound of 500 sq mm was formed Haemostasis was achieved by application of normal saline solution. The animals were kept in separate cages. The physical attributes of wound healing viz wound closure (contraction), epithelization and

Table No 1: Effect of gradient dose of ethanolic extract of *ficus retusa* by excision model

Post Wounding Days	Control	Standard framycetin 1%w/w	Extract 2%	Extract 5%
0	625	630	640	635
4	500	425	435	415
8	355	315	327	292
12	250	219	224	203
16	140	90	97	74
21	60	20	32	5

scar features were recorded. The wound contraction was studied by tracing the raw wound area on a transport paper on 4th,8th, 12th and 16th day and alternate The criterion for complete epithelization was fixed as formation of the scar with absence of raw wound area. The wound area was measured planimetrically by the help of sq. mm scale graph paper. The degree of wound healing was calculated as percentage wound closure area with the original wound area.

Incision Wound Model

Rats were divided in 4 groups of 5 animals each. The various groups were treated as follows:

The incision wound model was studied as described by Ehrlich and Hunt Under light ether anaesthesia the animal was secured to operation table in its natural position. Two paravertebral straight incisions of 6 cm each were made on either side of the vertebral column with the help of sharp blade. Incision was made at least 1 cm

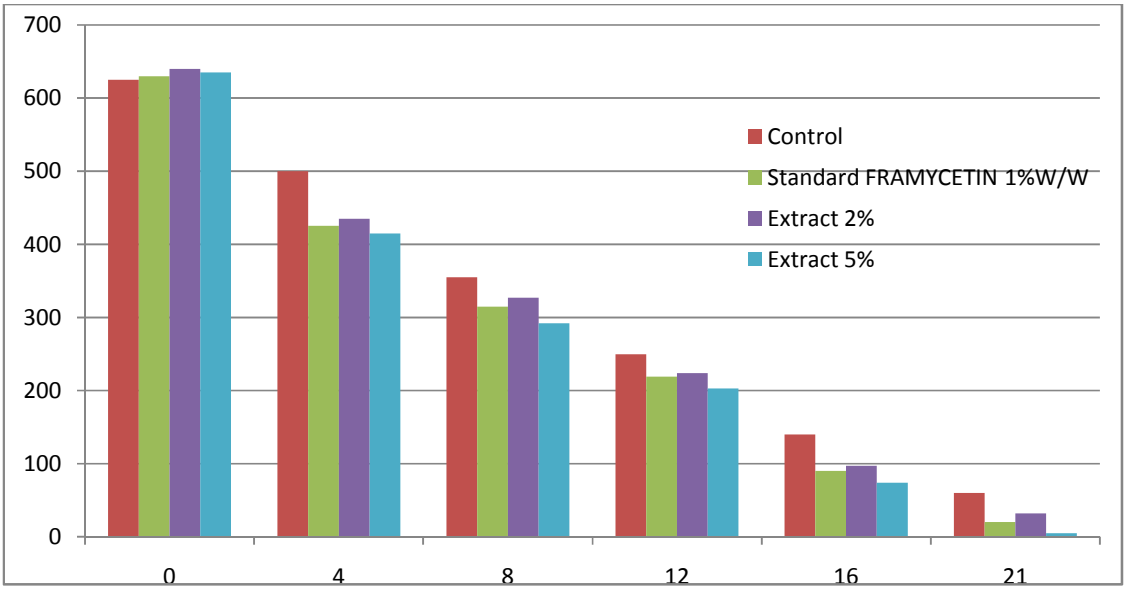

Fig. 1: Results from excision model determination

apart by silk thread of zero grades with the help of a straight round boided neddle. Wounds were cleaned with 70% alcohol soaked cotton swabs. The standard, control, and extract applied topically. They were kept in separate cages. The sutures were removed after 8 days. The tensile strength of the wound was determined on both sides by continuous constant water flow technique described by Lee In the continous constant water flow technique the anaesthetized animal was secured to opration table, the allis forceps were firmy fixed on the lines facing each other. The forcep on one side sis hooked to a meatel road to keep it in position while other forcep connected toa polythene reservoir by a string run over a pulley. The water was allowed to flow at a constant rate into polythene reservoir and the pulling force necessary to disrupt the wound was gradually built under controlled condition. The flow of water regulated with the help of an occlusion clam on polythene tubing was connected to the reservoir and was raised to a suitable height. As soon as the gaping of the wound was fromed the water flow was cut off. The pulling force of the wound was immediately released by lifting up the polythene reservoir to avoid further opening of the wound. The volume of water accumulated in the polythene reservoir was measured and was converted to corresponding weight by considering the density of water to be equal to one. The tensile strength was expressed as minimum weight of water necessary to bring about the gaping of the wound. Three such reading were recorded for a insicion wound and procedure was repeated on the other side, thus obtained total of 5 reading for each animal. The mean tensile strength of each animal was calculated by taking the average of five reading.

Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound healing agents promote a gain in tensile strength. The sutures were removed on the 9th day after wounding and the tensile strength was measured on the 10th day. The herbal ointment along with standard and control were applied throughout the period, twice daily for 9 days. The mean tensile strength on the two

Table No 2: Effect of gradient dose of ethanolic extract of ficus retusa incision model

Wound breaking strength	Control	Standard Framycetin 1%w/w	Extract 2%	Extract 5%
Tensile strength	407.14	819.78	619.57	1128.39

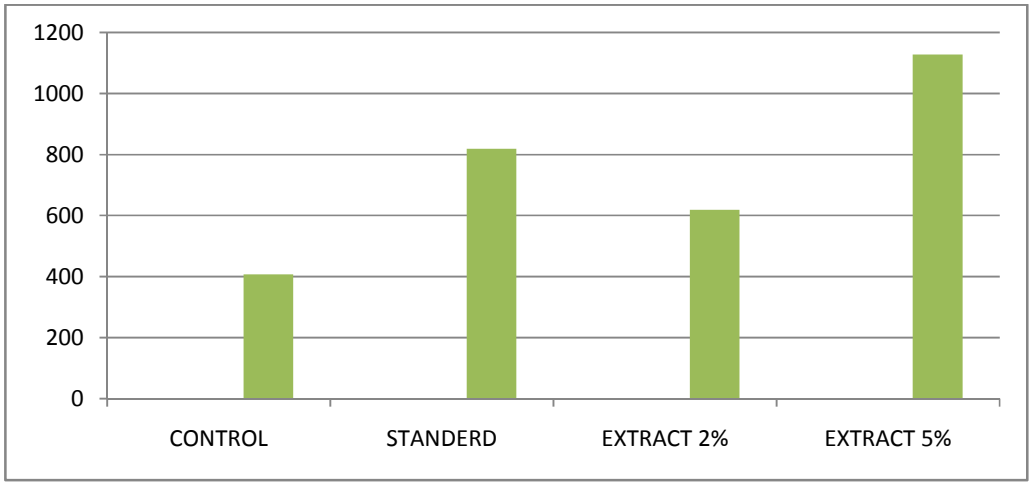

Fig. 2: Results from incision model determination

animals were taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of paravertebral incisions on both sides of the animals were taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of treated wounds was compared with control and framycetin 1%w/w as standard. The tensile strength increment indicates better wound healing stimulated by the applied herbal formulation. Further epithelization period and scar area were measured daily for 21 days after determination of tensile strength.

Summary and Discussion

The yield of ethanol extract of *Ficus Retusa* following removal of solvent and dry freeze. The results of the wound healing effects of the herbal extract in different concentration are shown in result. There was a general decrease in wound area upon application of the test samples and also with time. By the 21th day, the wound area of the animals that received the extract in (Group 2 and 4) were almost zero and by the 21th day, the wounds were completely healed. The wound healing activities of the extract in the different concentration are comparable and known antibiotic containing preparation with wound healing properties, by the 21th day. Results also show that the (Group 1 and 3) produced some degree of wound healing. The observed decrease in the wound area on the application of the herbal extract of the *Ficus Retusa* indicates that the plant extract possesses wound healing properties. The reduction in epithelization time of experimentally-induced wound by the extract of the plant has been described. *Ficus Retusa* was also reported to possess antimicrobial and haemostatic activities, which are necessary in wound healing process.

Since wound provide environment for microbial growth, the antimicrobial activity of the extract may partly contribute to the wound healing effect by eliminating infection thus allowing the natural tissue repair processes to start. It also suggests that the extract may play a useful role in accelerating the healing of old wounds by eradicating already established infection. The antimicrobial activity of *Ficus Retusa* is believed to be responsible for its usefulness in wound healing. The comparative wound healing effect of the extract and framycetin1%w/w suggests that antimicrobial effect of the extract plays a major role in its wound activity. Haemostasis involves the spontaneous arrest of bleeding from damaged blood vessels, which is important for initiation of tissue repair processes and prevention of tissue death through haemorrhage. The haemostatic process proceeds through a cascade of reactions, which starts with vascular spasm of the ruptured vessels, formation of platelet plug through platelet aggregation, and coagulation of the blood. The herbal extract may have facilitated these chains of haemostatic processes to effect the wound healing. Interestingly, the herbal extract alone showed some degree of wound healing effect indicating a possible synergy of the extract. The exact mechanism of such interaction is not clear. However, the interaction may have led to increased collagen formation since collagen is the principal component of any repaired tissue. In the tissue repair process, inflammatory cells promote the migration and proliferation of endothelial cells, leading to neo vascularisation of connective tissue cells which synthesize extracellular matrices including collagen, and of keratinocytes resulting to the re-epithelialisation of the wounded tissue. Additionally, the wound healing effect of the herbal extract could be attributed to the inherent ability to cover wound surface thereby eliminating infection and allowing the natural tissue repair process to take place. Furthermore, the wound healing activity of medicinal plants has been associated with their antioxidant properties. Tannins, the main components of many plant extracts, act as free radical scavengers and have been reported as partly responsible for the wound healing activity of *Ficus Retusa*. Other secondary metabolites such as saponins could be involved.

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