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EVALUATION OF WOUND HEALING ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *FICUS GLOMERATA* ON WISTAR ALBINO RATS

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Keywords:

Ficus glomerata, Wound healing, Anti-inflammatory, Excision, incision, Tensile strength

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ABSTRACT: Traditionally, *Ficus glomerata* is used for wound healing activity. Since no detailed scientific data are available regarding the wound-healing activity of *F. glomerata*, the present study was designed to explore the effect of hydro-alcoholic leaf extract of *Ficus glomerata* (HEFG) on experimentally induced excision and incision wound models in Wistar rats. The wound-healing efficacy of Hydroalcoholic extracts of *F. glomerata* was evaluated in excision and incision wound models by topically applying the Hydroalcoholic extract in the form of ointment as per Indian Pharmacopeia. The extract was formulated in ointment base at concentrations of 5% and 10% and topically applied to the wounds. In the excision and incision wound model, topical application of different concentrations of *Ficus glomerata* extract ointment (5% and 10% w/w extract in the simple ointment) has shown a high rate of wound contraction and decrease in the period of epithelisation time and improved the tensile strength to a greater extent when compared to control (simple ointment treated) group. The 10% extract ointment treated group demonstrated greater wound-healing promoting property than the 5% extract ointment treated animals. In the excision wound model, the animals showed a significant reduction in the period of epithelisation and improved wound contraction, whereas the increase in the breaking strength was evident from the incision model. The results suggest that the hydro-alcoholic leaf extract of *Ficus glomerata* (HEFG) possesses wound healing property when applied topically.

INTRODUCTION: Any damage to the cellular components or disruption in the anatomical as well as functional integrity of the skin is defined as wound [1]. The factors responsible for wounds vary greatly and generally include physical injury, chemical injury, and microbial infections. Wound healing is an outcome of tissue homeostasis and fundamental response to tissue injury [2].

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The healing process, which comprises of a series of events, usually begins from the very day of the injury and aims at maintaining the overall physical and functional integrity of the skin by process of connective tissue repair.

Coagulation, inflammation, granulation tissue formation, matrix formation, remodeling of connective tissue, collagenisation, and acquisition of wound strength are involved in the healing process, which can be categorized into the inflammatory, proliferation, and remodeling phases [3, 4]. Wound healing is mainly a process of a repair mechanism that recovers injury to the skin and other soft tissues. Due to injury, which is an inflammatory response occurs, and the cells below

the dermis (the deepest skin layer) begin to increase collagen production. The outer portion of the skin means the epithelial tissue is regenerated. Throughout the history of civilization, plants have served as a vital source of medicine to mankind for the treatment of various ailments. The active principles present in plants have been proven as therapeutically active agents [5]. These phyto-constituents have played various roles in the mitigation and management of numerous human diseases, and wound healing is no exception. The searches for efficacious such as wound healing have been an area of concern. Moreover, the adverse effects of synthetic drugs have insisted the researchers explore the potential of medicinal plants having wound healing activity. Worldwide research is going on the medicinal plant to find out the effective and less toxic phytoconstituents in the management of wound healing. However, many herbal agents that have been claimed to promote wound healing are yet to be scientifically screened and remains obscured. *Ficus glomerata* (Moraceae), a plant distributed in different parts of India, was evaluated for its wound healing activity. Traditionally the plant is reported to be used for the treatment of diarrhea, hemorrhoids, and diabetes skin disease [6]. A decoction of leaves of *Ficus glomerata* is used for washing wounds and ulcers [7]. Traditionally people are using the plant, but there is no scientific report on the effect of *Ficus glomerata* on wound healing. The present study aims to ascertain the effects of hydro-alcoholic leaf extract of *Ficus glomerata* (HEFG) on experimentally induced excision and incision wound models.

MATERIALS AND METHOD:

Drugs and Chemicals: The standard drug, Povidone-iodine ointment, was purchased from Unilab Chemicals & Pharmaceuticals Pvt Ltd, Mumbai, Maharashtra 400093, the ingredients for ointment, hard paraffin, Cetostearyl alcohol, etc. was purchased from S. D. Fine Chem. Ltd (Mumbai). All other chemicals used for this study were of analytical grade.

Plant Materials: Leaves of *Ficus glomerata* were collected during the month of April 2007 from the surroundings of Harpanahalli, Karnataka. The plant species were identified by Professor K. Prabhu, Dept of Pharmacognosy, S.C.S college of Pharmacy, Harpanahalli. A voucher specimen was deposited at the museum of S.C.S college of Pharmacy for future reference (SCSCOP/39/99).

Preparation of Extract: The leaves of *Ficus glomerata* were shade-dried and coarsely powdered. The powder was subjected to Soxhlet extraction and extracted with a hydro-alcoholic solvent (70% ethanol and 30% water) for 72 h. The excess of solvent was removed using Rotary Flask Evaporator, and the crude extract obtained was stored in airtight containers in a refrigerator below 10 °C for further studies. The yield (w/w) of the crude leaf extract of *Ficus glomerata* was 14.5%.

Preliminary Phytochemical Test: Phytochemical properties of the hydro-alcoholic residue of *Ficus glomerata* were tested using various reagent: Mayer and Dragendorff's reagent for alkaloids [8] $FeCl_3$ for tannins; frothing test for saponins; Magnesium chip and HCl for flavonoids [9] NaCl and Fehling's solution A and B for glycosides; diethyl ether, sulphuric acid and acetic anhydride for steroids; ether chloroform and NaOH for anthraquinone and $FeCl_3$ and $K_3Fe(CN)_6$ for phenols and polyphenols [10].

Animals: Wistar albino rats of either sex weighing between 150-250 g were used for the present study. The animals were obtained from the Sri Venkates wara Enterprise, Bangalore, and were maintained under uniform laboratory conditions at 27 °C ± 2 °C under 12 h light / dark cycle and housed in standard polypropylene cages. The animals were acclimatized for a period of 16 days prior to performing the experiments and fed with a standard rodent pellet diet (Lipton Gold Mohur, India Ltd, Bangalore) and had free access to water. Experiments were initiated only after the approval of the study protocol by the Institutional Animal Ethics Committee (IAEC- 157/1999 CPCSEA).

Preparation of Ointment: Test samples of the HEFG were prepared in an ointment base. The simple ointment was prepared, which consisted of weighed quantities of white bee's wax (2%), hard paraffin (3%), cetosteryl alcohol (5%), and white soft paraffin (90%) and melted. Weighed quantities of leaf extract of HEFG (5% and 10%) were mixed with the simple ointment by using an ointment slab and spatula. The formulated ointments were pre-served in the refrigerator for wound healing studies.

Experimental Design: The animals were numbered and divided into 6 groups with 5 animals in each group. The following groups were used in both the models and treated accordingly.

Group A: Control, applied topically 0.5 g, simple ointment.

Group B: Standard, applied topically 0.5 g, 5% w/w Povidone iodine ointment.

Group C: Treated with HEF G5% w/w ointment 0.5 g, topically.

Group D: Treated with HEFG 10% w/w ointment 0.5 g, topically.

Wound Healing Activity: Excision and incision wound models were used to evaluate the wound healing activities.

Excision Wound Model: The excision wound model was used to monitor wound contraction and closure time. The rats were slightly anesthetized with diethyl ether, and dorsal hairs were removed from the dorsal thoracic region of the rats using a hair removing cream purchased from the market (Veet manufactured by Reckitt Benckiser). An area of 500 mm^2 was marked on the shaved area with an indelible ink and rubber seal. The marked area was washed with normal saline and cut throughout the marked area through the skin to create a circular excised wound [11, 12]. The wounded rats were kept individually in separate cages with the wounds left undressed. The HEFG' ointment (5% and 10%) and the reference drug Povidone-iodine were applied topically once daily till the wound was completely healed. The wound area was studied by tracing the raw wound area on a transparent paper on 4^{th}, $8^{th,}$ and 12^{th} day, employing a millimeter-scale graph paper. The criterion for complete epithelisation was fixed as the formation of scar with the absence of a raw wound area. The percentage of reduction in the wounded area was calculated by using the following formula.

Percentage of wound contraction = (Wound area on day "0"- n (wound area on days) / (Wound area on day "0") × 100

Where, *n* = number of days (4^{th}, 8^{th} and 12^{th} day).

Incision Wound Model: In the incision wound model, the ether anesthetized rats were incised with two longitudinal paravertebral incisions of 6 cm length [13.] Incisions were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back. Using surgical thread (No. 000) and sterilized curved needle (No. 9) the incised parted skins were sutured, each suture separated with 1 cm gap.

The undressed wounds were treated with topical application of the ointments as described above for a period of 10 days. The wounding day was considered as day '0'.The sutures were removed from the healed wounds on the 8^{th} post-wound day. The tensile strength was measured on the 10^{th} day using a tension-meter. By the application of the continuous water flow technique, the breaking strength of the wounds was measured and expressed in Newton. In other words, the tensile strength of the skin is the weight required to break or open the wound, and the same was measured on the 10^{th} day [14.]

Statistical Analysis: All the results were shown as Mene ± SEM. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by the Turkey Krammer Multiple Comparison Test. * p < 0.0 5, **p < 0.01, * *p < 0.001 was considered as statistically significant.

RESULTS:

Phytochemistry: Preliminary phytochemical screening showed the presence of flavonoids and tannins as the major phytoconstituents in the hydo-alcoholic extract of the leaves of *Ficus glomerata* (HEFG).

Excision Wound model: In the excision wound model, topical application of different concentrations of HEFG extract ointment (5% and 10% w/w extract in the simple ointment) demonstrated a high rate of wound contraction and decrease in the period of epithelisation time when compared to control (simple ointment treated) group.

The 10% extract ointment treated group demonstrated greater wound-healing promoting property than the 5% extract ointment treated animals Result are shown in **Table 1**. However, the result indicated that wound healing potency of the 10% extract ointment was lesser than the reference standard (5% povidone-iodine) ointment.

TABLE 1: EFFECT OF TOPICAL APPLICATION OF OINTMENT CONTAINING HYDROALCOHOLIC LEAF EXTRACT OF *FICUS GLOMERATA* ON EXCISION WOUND PARAMETERS

S. no.	Treatment	Mean Percentage of Wound Contraction ± SEM				Period of Epithelisation (days)
		4th day	8th day	12th day	16th day	
1.	Control	15.76± 3.00	30.94± 2.50	44.87± 3.20	61.67± 4.21	23.5 ±0.63
2.	Standard povidone iodine 5 %	33.51±2.64***	59.93±3.38***	88.19±4.10***	96.61±3.73***	18.00±0.54***
3.	*Ficusglomerata*5%	25.45±2.00*	44.28±2.60*	53.89±3.65**	71.49±3.29**	20.80±0.68*
4.	*Ficusglomerata*10 %	28.34±2.77**	47.00±2.57**	62.67±3.82***	86.60±4.97***	19.60±0.67**

The values are expressed as Mean ± SEM, n=6 in each group. * P<0.05, **P<0.01 and ***P<0.001, when treated groups compared with the normal control group.

Incision Wound Model: In the case of the incision wound model, topical application of HEFG ointment showed a significant increase in the skin breaking strength than the control group. A higher dose of test extract ointment improved the tensile strength to a greater extent than the lower dose results are shown in **Table 2**. However, both the test doses were found to be less effective than the reference standard, Povidone-iodine ointment.

TABLE 2 EFFECT OF OINTMENT CONTAINING HYDROALCOHOLIC LEAF EXTRACT OF *FICUS GLOMERATA* ON BREAKING STRENGTH (G) IN INCISION WOUNDS

S. no.	Group	Tensile Strength in Grams (Mean ± SEM)
1.	Control	430.4 ± 6.46
2.	Standard Povidone iodine 5%	571.0 ± 8.84**
3.	*Ficus glomerata* 5%	467.9 ± 8.96*
4.	*Ficus glomerata* 10%	481.2 ± 7.15**

The values are expressed as Mean ± SEM, n=6 in each group. * P<0.05, **P<0.01 when compared to control group.

DISCUSSION: The process of tissue repairing involves a series of events, migration of inflammatory cells, and proliferation of endothelial cells leading to neovascularization of connective tissue cells that synthesize extracellular matrices including collagen resulting in re-epithelialization of wounded tissue [11].

Sometimes the natural process of healing is delayed due to exposure of the wound to the environment leading to infection and other complications. The aim of wound healing is first to improve the rate of healing process, causing minimal pain and reducing the probable complications [14].

The results of the present study indicated that 70% hydro-alcoholic leaf extract of *Ficus glomerata* (HEFG), when applied as topical ointment at both strengths (5% and 10%), exhibited significant wound healing promoting activity. However, this effect was found to be in a concentration-related fashion. This was evident by a faster rate of wound closure and epithelisation period in the excision wound model and a significant increase in the skin breaking strength in the incision wound model. Flavonoids and tannins were found to be the major phytochemical constituents in plant extract as per the phytochemical results. Several studies conducted in the past have revealed that flavonoids and tannins have the potential to promote the process of wound healing. The Probable factor for wound contraction shown by flavonoids might be due to its astringent and antimicrobial property. Where Flavonoids increase the rate of epithelisation, which substantiates its uses as wound healing promoter [15], tannins are responsible for various cellular processes like chelation of reactive oxygen species and other free radicals, acceleration of capillary vessels formation, and promoting the wound contraction [16]. The function and recruitment of various inflammatory cells, fibroblasts, and keratinocytes which is effected by the stimulation of interleukin -8 an inflammatory alpha chemokine, may increase the gap junction intracellular communication in cultured fibroblasts. This induces a more rapid maturation of granulation tissue, thus promoting the contraction of wounds [17]. The increase in cellular proliferation and collagen synthesis at the wound site was evident from the increase in wound breaking strength in the incision wound model at higher concentrations. In incision wound, the increase in

tensile strength of skin in treated wounds may be due to an increase in collagen concentration and stabilization of fibers [18]. The results of the present study indicated that 70% hydro-alcoholic leaf extract of *Ficus glomerata* (HEFG), when applied as topical ointment at both strengths (5% and 10%), exhibited significant wound healing promoting activity. However, this effect was found to be in a concentration-related fashion. This was evident by a faster rate of wound closure and epithelisation period in the excision wound model and a significant increase in the skin breaking strength in the incision wound model.

CONCLUSION: The findings of the present study have proved the beneficial effects of *Ficus glomerata* in excision and incision models of wound healing. From the observations and results obtained in this study, it can be inferred that the significant wound healing potential of HEFG may be due to flavonoids and tannins content, which were confirmed by preliminary phytochemical screening. Further, research is required in this aspect to isolate the active compounds in order to understand the molecular mechanism. Never the less, the present findings could justify the inclusion of the *Ficus glomerata* as an adjuvant in the wound healing therapy.

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